JOURNAL OF AGRICULTURAL RESEARCH

VOL. 52 WASHINGTON, D. C., JANUARY 15, 1936 NO. 2

MODIFICATION OF A MENDELIAN RATIO IN MAIZE BY POLLEN TREATMENTS [1]

By J. H. KEMPTON

Botanist, Division of Genetics and Biophysics, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Of the millions of pollen grains produced by a single maize plant (*Zea mays* L.) not many more than 1 in 25,000 effect fertilization; the others perish. To a large extent chance determines which grains function and which are wasted, but survival or demise is not wholly a matter of chance. An element of selection intervenes, resulting in a differential survival value of certain sorts of pollen grains.

Some pollen grains bear within them a hereditary pattern that precludes functioning irrespective of external agencies. Others have a genetic complex that enables them to survive certain unfavorable environments. An example of the latter class is found in the so-called waxy gene—the hereditary element that produces a form of starch giving a red instead of a violet reaction with iodine. Pollen grains possessing the waxy gene, and in consequence having red-staining starch, sometimes survive conditions that are lethal to grains containing the form of starch normal to commercial varieties.

Some years ago, in the course of an investigation of the factors affecting variability of cross-over ratios in maize, it was unexpectedly discovered that the proportion of functioning pollen grains bearing the waxy gene to those bearing its dominant allelomorph could be profoundly altered by storing the pollen for several hours in the sunlight. Storage for the same length of time in the dark was ineffective.

This phenomenon was verified for 3 successive years, while attempts were made to standardize treatments and develop a technique that would result in a uniform reaction. Satisfied by these early experiments that the observed results were real, a project was launched in an attempt to determine the causal factors that operated during the storage period. The results were disconcerting in that the effects of many of the treatments contradicted the work of previous years.

Consequently a return was made to the original technique, with the surprising result that treatments formerly effective in increasing the proportion of functioning waxy [2] pollen grains were found now to be

[1] Received for publication Sept. 19, 1935; issued March 1936.

[2] The two sorts of pollen grains dealt with in this study are designated "horny" or "waxy" depending upon which member of the allelomorphic pair *Wx*, *wx* they bear. No ambiguity should result from this practice, and by this means the awkward expressions "waxy bearing" and "nonwaxy bearing" are avoided while the advantage of contrasting names is obtained.

lethal. Pollen exposed to direct sunlight for periods even as short as 10 minutes was completely devitalized.

The accumulating inconsistencies, probably rather more than the merits of the problem itself, stimulated further investigation and prolonged the study. Much remains to be done before a complete knowledge is attained of the factors that bring about the differential functioning of these two sorts of pollen grains. Definite evidence has been obtained that even with genetically uniform stocks the pollen of some plants behaves differently under adverse treatments from that of others. Clearly the conditions under which pollen is produced are important factors in determining its subsequent reaction. Since the conditions operating throughout the maturing of pollen are largely beyond artificial control it is evident that physiologically identical pollen samples are not to be had from place to place or from season to season.

It is still not possible to outline a treatment that will produce a predictable alteration in the proportion of waxy to horny functioning pollen grains. However, some of the difficulties encountered in these investigations and the means employed to surmount them may be of interest to those undertaking similar studies in the physiology of pollen.

In the course of this study extensive data have been assembled, and it is the purpose of the present paper to summarize this accumulated information.

REVIEW OF LITERATURE

The literature on the effect of pollen storage on Mendelian ratios is brief, but several papers on gametic selection have a direct bearing on this subject.

Knowlton (*13*),[3] in a study of pollen longevity, found that the maximum duration of fertilizing power of pollen of Golden Bantam sweet corn was from 70 to 80 hours. He concluded that the death of corn pollen is caused by desiccation but pointed out that moisture is not the only important factor since life is not greatly prolonged by storage under conditions which retard evaporation. He failed to obtain seeds with pollen stored over calcium chloride at a temperature of 34° C., but his shortest period of storage in this experiment was 6 hours.

In several of Knowlton's experiments the percentage of functioning grains was higher after a long period of storage, as, for example, when pollen was stored at a humidity of 80 to 90 percent at 20° C. With pollen treated in this manner he obtained the following percentages of fertilization: 24 percent at 6 hours, 67 percent at 24 hours, 82 percent at 30 hours, 5 percent at 36 hours, 23 percent at 48 hours, and 6 percent at 54 hours. These percentages were obtained by counting the fertilized and unfertilized ovules in each ear, but only the number of ears is given. In the experiment just mentioned, two ears were used for each period with the exception of the 48-hour period, when three ears were pollinated, and of the 54-hour period, in which only one ear was pollinated. From the published data no estimate of errors is possible, and probably none is justifiable because of the great variability, but it is of interest to observe that apparently certain storage conditions are favorable to longevity of corn pollen.

[3] Reference is made by number (italic) to Literature Cited, p. 120.

Knowlton (*13, p. 787*) states:

> The results from the storage experiments with pollen were exceedingly variable. Many factors that are difficult to control may influence the results. Pollen produced in different seasons and under diverse conditions is, in some way, physiologically different, as is shown by differences in water content, the different optimum sugar concentrations required for germination, and the variation in duration of life.

Holman and Brubaker (*11a*) tested the longevity of pollen of about fifty species when stored in the dark under various degrees of humidity. Their measure of vitality was the ability of the grains to germinate in sugar solutions. Certain difficulties were encountered in obtaining uniform samples of pollen, which they believe could be obviated by mechanical mixing. However, they noted with several species that the lots of pollen contained some grains much longer lived than others. They state:

> In brief, it may be said, then, that in the case of many pollens each lot consists of a relatively large number of grains which retain their viability for a relatively short period, and a smaller number, constituting generally less than 10 percent of the whole, which survive storage several times as long as the others.

They note also that the pollens of the grasses differ from those of most other plants tested in that the death of grass pollen is caused by the loss of water, whereas lowering the water content extends the life of many other plants.

Brink (*3*) reports unpublished experiments of Thelma McWilliams on pollen storage that not only failed to increase the proportion of functioning waxy pollen but in fact showed the opposite effect. In these experiments aging pollen tended to lower the proportion of effective waxy pollen grains.

Sprague (*21*) investigated the time required for germination by different sorts of maize pollen on excised silks. He found that on the average waxy pollen grains required 3.8 minutes longer for germination than did the horny pollen grains. This time differential was very much greater when the pollen grains were carrying the sugary gene in addition to the waxy one. It is quite possible that the effects observed from pollen storage are brought about by changing the time required for germination by different sorts of pollen grains.

Ciesielski (*6*), working with hemp (*Cannabis sativa* L.), conducted experiments on the alteration of the sex ratio resulting from the use of stored pollen. After finding a minor difference in the ratio between seeds resulting from early morning pollinations and those from pollinations made in the afternoon, he tried storing pollen in paper. He states (*6, pp. 10–11*):

> In view of the success of these experiments in fertilization I took up, in 1877, six female plants with their roots, before they had fully produced their flowers, and transplanted them into pots: as soon as the plants were well-rooted I transferred the pots to the windows of two rooms looking south, three pots in each room. Thereafter, with a brush, I fertilized the three plants in one room with pollen which I collected from anthers just dehiscing and not yet fully open (this pollen I shall refer to as "fresh"), and the three pots in the other room I also fertilized by means of a brush, but with pollen taken in the morning and kept in paper till evening. The three plants fertilized with "fresh" pollen produced 120 seeds; the three fertilized with "stale" pollen produced 96 seeds.
>
> In 1878 I sowed in my garden with great care the seeds of the two lots separately, at distances of 20 centimetres. The 120 seeds derived from fertilization with fresh pollen produced 112 plants, of which only six were female, all the rest being male. But the other 96 seeds, sprung from fertilization by stale pollen, produced 89 plants, every one of which was female. I have several times since repeated the experiment, always with a similar result.

The actual experiment reported leaves no room for doubt as to the different sex ratio in the two lots of seed, but since no mention is made as to the common source of the pollen it does not follow that the results are due to the effects of storage. However, in view of the statement of the results of repetitions, there is little reason to question the effects of pollen storage on the sex ratio in hemp.

Mangelsdorf (*16*, *17*) has shown that certain types of maize pollen can be sorted mechanically by means of bolting sieves. Although size differences in pollen grains have no direct bearing on storage, the fact that such differences do exist must be taken into account in sampling.

Bond (*1*), working with shape and color of seeds in peas, has shown that the Mendelian proportions of these characters are altered by pollen storage, although he made no direct storage experiments. In his investigations pollen was applied to mature and immature stigmas. That on the immature stigmas presumably was stored until the stigmas were receptive.

Christie and Wriedt (*5*) found a seasonal variation in the Mendelian ratios of certain color factors in pigeons and chickens, but they failed to discover any seasonal effect on the ratios of yellow and green or tall and dwarf peas.

Correns (*8a*) investigated the effect of aging pollen on the sex ratio in *Mercurialis*. Pollen was stored for various periods up to 120 days, and the seeds resulting from the treated pollen were planted with a control obtained by the use of fresh pollen. In general it was found that the older the pollen the higher the proportion of male to female plants in the progeny.

Malhotra (*14*, *15*) showed that the sex ratio in asparagus could be altered by pollen storage. Certain treatments increased the ratio of male to female plants whereas other treatments reversed this effect.

Sisa (*20*) found that pollen vitality in cucurbits changed rapidly with age. He observed that the germinating power of pollen in flower buds increased from early morning until 9 a. m. of the day preceding blooming. From this time until the flowers opened the germinating power decreased slowly, but at 10 a. m. of the day of blooming there was a sudden decrease in pollen vitality.

Nakamura (*18*) records a remarkable fluctuation in the occurrence of imperfect pollen grains in *Impatiens balsamina* L. associated with changes in the air temperature. He found that imperfect pollen grains varied from 9 to 100 percent, the imperfections being due to irregularities in meiosis. Normal cell divisions took place when the maximum air temperature was less than 30° C., but at temperatures above this point there is nonconjunction, nondisjunction, lagging, grouping, etc. Irregularities of this nature are not present to complicate the problem of differential functioning of maize pollen. Classification of pollen samples by staining with iodine shows great regularity in the ratio of waxy to horny grains, and it must be concluded that these two sorts of pollen are formed in the expected proportions.

Haigh and Lochrie (*11*), in a study of seasonal effect on Mendelian ratios in *Vigna*, found a strong tendency for the plants to produce an excessive proportion of recessive forms in the first flowers and a corresponding excess of the dominant forms in the last flowers.

Sasahi (*19*) cultivated pollen in various nutrient media and also studied how long it would retain its power to form pollen tubes. He

concluded that maize pollen was preserved best at 50 percent humidity. He found that the duration of the preservation of fertilizing power does not always coincide with the preservation of the ability to form pollen tubes and concluded that the duration of the real fertilizing power must be determined by actual pollinations.

Gotoh (*10*) shows there are many factors that influence germination in artificial media. Quality of the glass, hydrogen-ion concentration of the medium, and dryness of the pollen are all important factors. Bursting is influenced more by the pH of the medium than by osmotic pressure. He found that different varieties of maize vary greatly in the percentage of pollen grains that will germinate. He found also that pollen with from 10 to 50 percent of shrunken grains germinated better than turgid pollen and that the highest percentage of germination occurred near the point of greatest bursting.

METHODS

As this investigation progressed, several methods of treating pollen during storage were tried. These included exposure to direct sunlight for various time intervals and to artificial light of several intensities, storage in high and in low temperatures, storage in atmospheres of oxygen and of carbon dioxide and storage for 17 and for 24 hours in the dark.

Rather early it became apparent that the question of uniform sampling might be important. Accordingly an attempt was made to determine whether random sampling could be achieved in composite samples and further to analyze the factors that might affect subsampling. By means of an air blast in vertical long glass tubes, an attempt was made to discover whether the two sorts of pollen grains differed in weight; fine wire bolting sieves were used in an effort to determine differences in size, and specific gravity separations were made with solutions of alcohol and chloroform. In these experiments the sorted pollen grains were classified microscopically and duplicate samples were used in pollinations as a check on differential functioning.

In the earlier experiments all pollinations were made on an individual plant basis. Pollen was collected separately from heterozygous plants, and the identity of each sample was retained for all treatments. This procedure involved a control pollination for each plant furnishing pollen, a practice that doubled the work not only in making and recording pollinations but also in the subsequent classification of seeds. There was a further disadvantage of this system in that many of the pollen treatments proved lethal and their control ears from fresh pollen were consequently useless.

To obviate these disadvantages bulk samples of pollen were collected from as many as 50 heterozygous plants. The samples were sieved to remove anthers, then thoroughly mixed by pouring back and forth, and subsequently divided into as many subsamples as the quantity permitted. Care had to be exercised in mixing, as the grains tended to clump and even to become pasty if too large quantities were collected and the mass became too dense.

This method of handling pollen simplified field practice, but with the exception of a single small group all the ears resulting from the use of bulk pollen samples showed significant reductions in the pro-

portion of waxy to nonwaxy seeds irrespective of pollen treatment. It seemed reasonable to ascribe this unexpected result to some condition arising in the method of sampling, and until this possibility could be investigated the method of bulk sampling was abandoned. Subsequent attempts to obtain low percentages of waxy seed from bulk pollen samples have been unsuccessful, and the earlier results remain without satisfactory explanation.

Not only did the screening of numerous individual samples consume much time, but in many cases the accumulation of large numbers of damp anthers reduced materially the quantity of usable pollen, besides introducing a possible source of variability by affecting the moisture content of the pollen grains.

In the work of the last few years the tassels of pollen parents were enclosed in two bags, one within the other. The inner bag, slightly smaller than the outer one, was perforated with numerous fine holes too small to permit the passage of anthers. By this means the pollen sifted into the outer bag almost as soon as shed and no sieving was required. This pollen was taken from the field in tassel bags, transferred to small pill boxes, and given treatments while in these boxes. For pollinating the covered boxes were taken to the field, and in most cases two ears were pollinated from each box.

All female parents had their tassels removed and the silks were cut back 24 hours before pollination to provide uniform brushes of silks. Many days before silking, the ears were covered with open-ended bags closed at the outer end with flexible metal strips and closely fastened to the plant with copper wire. The technique employed on the female plants has not varied over the period of this investigation, and from the hundreds of seedless cobs obtained from many of the pollen treatments it was apparent that the error due to contamination was gratifyingly low.

Several stocks of waxy were used, but all of these have been in cultures of the Bureau of Plant Industry for many years and their behavior under ordinary conditions is well known. In the last 3 years this investigation has been limited to one stock, chiefly because that stock possessed large seeds capable of rapid classification. In the earlier work multiple-eared stocks with numerous small seeds were used, but since analysis showed no influence of the female parent and since the chances of errors in bookkeeping, to say nothing of the failures of lower ears to produce seed, were greater in these strains, they were discarded.

The experimental work was carried on at the Arlington Experiment Farm, Rosslyn, Va., and at Lanham, Md. This fact is mentioned, as the results from the two places were not always alike even in the same year. No question of seed stocks nor of personal equation is involved, however, as the seed and the personnel were the same in both localities. The geographical separation of the experimental work adds one more complication to the presentation of data already complex because of numerous time intervals, sampling techniques, and types of control.

ANALYSIS OF SAMPLING

The results of the effect of storage on Mendelian ratios in functioning pollen depend for their accuracy on the possibility of subdividing large samples of pollen into smaller subsamples.

Where pollen is collected in bulk from 25 or more heterozygous plants and is subsequently subdivided into subsamples for various treatments it is essential that the subsamples be alike at the start of the treatments.

Similarly, where pollen is collected separately from individual plants and this pollen is subdivided into lots, one to be applied as a fresh control and the others to be subjected to different treatments, the several lots must be random samples of the original collection.

After treatment there is no method for determining whether or not the control and treated subsamples were alike at the time of subdivision. It can be determined that an array of ears obtained from a given lot of pollen used fresh is a random one, and it may be assumed that the corresponding lots in the treated series were random also; but this does not provide a measure of a possible selection. It is quite possible in pouring subsamples to obtain two sets, both distributed at random about their respective means; yet the two means may differ unless care has been exercised to draw the samples for each set at random.

To provide a measure of the randomness of subsampling, each sample of pollen has been used to pollinate two ears. As is to be expected, especially where adverse treatments were given the pollen, there were many cases where only one ear of the pair produced seed. However, enough cases of paired pollinations were obtained to permit a measure of the randomness of subsampling. To these may be added certain ears where the butts and tips were classified separately.

In one experiment a bulk sample of pollen was collected from about 25 plants, thoroughly mixed, and subdivided into 10 lots of approximately equal size, which were subjected to a secular selection by the following method: The 10 subsamples were poured from a bag containing the bulk sample; the first subsample was numbered 1, and the last lot of pollen to leave the bag was numbered 10. These lots were used to pollinate 20 ears, each lot being used on 2 ears. The subsamples were applied consecutively, lot 1 being applied to ears *a* and *b*, lot 2 to ears *c* and *d*, etc. By this means it was hoped to obtain some knowledge as to whether or not the method of pouring subsamples involved an unconscious sorting of the pollen grains.

Only 17 of the 20 pollinations were effective. The correlation between the number of a subsample and the percentage of waxy seeds obtained is $\rho = -0.123 \pm 0.169$, clearly indicating that the method of sampling did not introduce any regular selection of the pollen grains.

When the variance of the population of paired ears resulting from the use of fresh pollen is subdivided into its several elements it is found that there is no difference between subsamples in the proportion of waxy to horny pollen grains that function in the production of seed. There is a correlation of 0.5 between the pollen grains within subsamples, but the number of samples is too small to attach any significance to the coefficient. The apportioned variance for this population is given in column 2 of table 1.

As a comparison with the above population, the variance is apportioned for two similar populations of paired ears resulting from the use of fresh pollen, where the pollen, instead of being collected in bulk from many plants, was collected separately from each plant and each collection was used on a single pair of ears. The mean squares for these two populations are shown in columns 9 and 10 of table 1.

It will be observed that the mean square of the means of subgroups is but two-thirds of that found when the bulk sample of pollen was used.

It was not to be expected that the mean square of the means of subgroups from pollinations with individual plant samples would be lower than the mean square of the means of subgroups from pollinations with subsamples of composite collections. Composite samples never should have mean squares larger than those of the individual samples, and the expectation would be for lower mean squares. Since the difference between the mean square for the composite sample (12,043.74) and that for the combined populations in columns 9 and 10 of table 1 (8,012.68) is not significant, the result may be attributed to chance.

Although in both populations from individual plant samples the mean square of the means of subgroups exceeds that of the pair members from their mean, the differences are not significant, and it may be concluded that for these populations there is no reason to believe that the ratios of the two kinds of pollen grains were more nearly the same within subsamples than between subsamples.

Three other populations resulting from the use of fresh pollen are available for analysis. In these three populations the subsamples consist of the butts and tips of the ears. Two of the populations are from the use of composite collections of pollen, the third being from individual plant pollinations. No manual division into subsamples is involved in these three populations, and in the population from individual plant pollinations the mean square of the means of subgroups measures the differences between male parents.

The mean squares are given in columns 6, 7, and 11 of table 1. In all three populations the mean square of the means of subgroups exceeds that of the members of the pair from their mean, and in two of the populations the differences are clearly significant. In the third (column 6) the difference just borders on significance. Were the comparison limited to the population given in column 11 it would be a fair inference that male parents differed one from the other, but with the composite samples no such interpretation is possible.

For purposes of comparison a single population has been included (column 13) where the variability is limited to the ovules, the pollen having been all of one kind—waxy. In this population the mean square of the means of subgroups (composed of butts and tips) is actually less, though not significantly so, than that of the butts and tips from their mean. This is the only case of the 12 presented in table 1 where the means of subgroups are less variable than their components. It is clear that the difficulties in sampling and the observed correlations between butts and tips and between paired ears from the same pollen subsample are confined to the pollen. Since the composite samples are less satisfactory in this respect than the individual plant samples, it is evident that the results flow from physiological rather than genetic differences in pollen. This conclusion receives further support when the populations of ears resulting from the use of stored pollen are examined.

There is one population resulting from the use of stored pollen collected from individual plants. The mean squares for this population are given in column 12 of table 1 and may be compared directly with those in column 11, which are for a population from the same

TABLE 1.—*Apportionment and analysis of variance of percentage of waxy, showing the mean squares for the several groupings*

Variation	Composite pollen sample split into subgroups							Individual pollen samples collected from separate male parents				
	Pollen, after indicated treatment, applied to paired ears				Ears resulting from pollen used after indicated treatment, classified separately for butts and tips			Fresh pollen applied to paired ears		Ears resulting from pollen used after indicated treatment, classified separately for butts and tips		
	Fresh	Stored at 37° C.[1]	Stored at 10° C.[1]	Stored in oxygen [1]	Fresh Lanham 1929	Fresh Arlington 1929	Stored in sun 5 hours			Fresh	Stored in sun 7½ hours	Male waxy, female heterozygous
1	2	3	4	5	6	7	8	9	10	11	12	13
Apportionment:												
Total seeds.....mean square	2,488.31	2,160.98	2,143.24	2,340.42	2,500.20	2,465.05	2,482.46	2,477.11	2,473.27	2,459.82	2,497.39	2,500.04
Seeds on ear.....do	2,474.48	2,154.51	2,133.13	2,339.02	2,486.96	2,455.82	2,464.77	2,465.98	2,464.03	2,442.98	2,433.87	2,498.24
Ears.....do	8,021.81	5,444.39	6,757.41	3,038.91	6,031.29	4,824.11	7,430.89	7,328.34	7,865.02	7,111.36	19,669.99	2,929.06
Pair members from their mean.....do	4,574.43	3,841.87	3,227.89	2,606.18	3,205.91	77.20	1,734.30	7,201.34	7,465.04	2,789.93	6,126.19	3,027.46
Means of subgroups.....do	12,043.74	7,848.15	12,492.90	3,687.99	9,092.13	10,363.17	13,443.96	7,468.03	8,285.00	11,576.83	33,460.03	2,879.78
Analysis:												
$\frac{\text{Means of subgroups}}{\text{Pair members from their mean}} = z$.....	.483	.356	.678	.174	.520	2.43	.102	.018	.052	.709	.845	.024
Significance of z.....P	>.05	>.05	>.01	>.05	>.01	<.01	<.01	>.05	>.05	<.01	<.01	>.05
Primary groups.....number	14	11	22	16	26	14	38	22	42	62	112	236
Total seeds.....do	5,216	7,780	9,609	7,501	6,694	3,336	10,385	9,179	23,952	16,912	30,120	59,550
Waxy seeds.....mean percent	46.50	31.58	31.11	37.36	50.42	44.03	45.78	45.19	44.80	43.65	48.36	49.96

[1] Stored for 2½ hours and applied 3½ hours after collection.

pollen parents where the pollen was applied immediately following collection. The enormous increase in the mean square between ears will be noted in this population of ears from stored pollen. This may mean either that the stored samples of pollen have not been accorded uniform exposure to sunlight or that the samples reacted differently to exposure. Probably the high intraclass correlation ($\rho=0.701\pm0.046$) is the result of both factors. It should be noted, however, that the mean square of the pair members from their mean is also excessive in this group of ears. Thus the tips and butts of the same ears tend to differ from each other, indicating a differential functioning of the two sorts of pollen tubes in long and short styles.

A similar situation is encountered in a population of ears resulting from the use of pollen stored in the sun where a composite collection of pollen was subsampled. The mean squares for this population are given in column 8 of table 1.

In columns 3, 4, and 5 of table 1, the mean squares are given for a single composite sample of pollen, which was subdivided into small samples that were used in each case to pollinate a pair of ears. All three lots of subsamples were drawn at random from a single bulk collection, treated for 2½ hours, and applied 3½ hours after collection. In all three cases the waxy percentages are low although the lots stored at 37° and 10° C., respectively, do not differ significantly, the χ^2 of the difference of these two populations being 3.31 ($P=0.069$), The samples stored in oxygen have a significantly higher percentage of waxy seeds than either of the two temperature series. The oxygen treatment differs from the 37° treatment, with a χ^2 of 52 ($P<10^{-8}$), and from the 10° group, with a χ^2 of 97 ($P<10^{-8}$). The oxygen series shows rather conclusively that the subsampling was random, whereas the 10° treatment shows evidence of an intraclass correlation.

If the 37° and the 10° C. populations are combined, on the assumption that the storage treatments resulted in no differential behavior, and the variance of this combined population is apportioned, it is found that the mean square of the means of pairs is 10,477.58 and that of the pair members from their means is 3,420.78. The ratio of the two is 3.062, with P lying between 0.01 and 0.05. There is, therefore, just a possibility that the pairs of pollinations in this temperature series are not random subsamples of the original composite sample.

Further analysis of variance is possible in the case of a single population where ears were pollinated in pairs and the butts and tips of each ear were classified separately. The pollen in this case was a composite sample split into small subsamples and these were stored in sunlight for 5 hours. Each subsample was applied to two ears. The mean squares for duplicate samples used fresh are given in column 7 of table 1. Not all the paired pollinations were successful, and the mean squares for all the ears irrespective of pairs are given in column 8.

Limiting the population to the ears where paired pollinations were successful and apportioning the variance into its component elements, it becomes possible to compare the variance of subgroups based on butt and tip sampling with the variance of subgroups composed of paired ears.

The data are as follows:

Apportionment of variation:	*Mean square*
Total seeds	2, 467. 89
Seeds on butts and tips	2, 458. 60
Seeds on ear	2, 457. 08
Butts and tips	5, 172. 66
Ears	8, 802. 46
Butts and tips from their mean	1, 845. 34
Means of subgroups of butts and tips	8, 802. 46
Ears from their pair mean	5, 900. 78
Means of subgroups of paired ears	12, 284. 48
Analysis of variation:	*Number*
Primary groups of butts and tips	24
Subgroups of butts and tips	12
Primary groups of whole ears	12
Subgroups of paired ears	6
Total seeds	6, 456
	Mean percent
Waxy seeds	44. 3

Comparing the mean squares of this restricted population with those obtained for all the ears as given in column 8 of table 1, it is seen that the agreement is fairly close in all categories other than the mean squares of the means of subgroups composed of butts and tips. In the unrestricted population this is 13,443.96, and in the limited population it is only 8,802.46. The difference is not significant, but apparently in limiting the population to paired ears some of the variability was eliminated. In the restricted population it is seen that the intraclass correlation between butts and tips is significant, z equaling 0.78 and P being less than 0.01. No such correlation can be demonstrated for the paired ears, even though the mean square of the means of subgroups exceeds that found for the means of subgroups composed of butts and tips. The mean square of the individual ears from their pair mean is also larger than the mean square of butts and tips from their mean, and z equals 0.367, P being greater than 0.05. The difference between the two intraclass correlations, as measured by z, is 0.415 ± 0.398.

From the analysis of the variance of the percentage of waxy seeds in butts and tips of these ears, it is clear that the variation between butts and tips is much lower than that between individual ears despite the fact that the pollen was a composite sample. No such condition can be demonstrated between paired pollinations, although there is evidently a tendency in this same direction since the mean square of the means of pairs is twice that of the members of the pair from their mean. Just why the butt and tip of an ear should be more nearly alike than a pair of ears pollinated with the same pollen is not clear. It might be urged that this evidence indicates an influence of the female parent, which, of course, in these cases, was homozygous for waxy and could not contribute directly to variation in waxy percentage unless the hypothesis of selective fertilization is invoked. Other evidence of the influence of the female parent is discussed later.

Not only is the variance of the means of paired ears relatively high, but the variance of the butts and tips from the mean of the ear is too low, clearly indicating an ear individuality.

From a consideration of these mean squares for the several sorts of groupings it is apparent that there is a real problem of subsampling

involved in these experiments, and it is indicated further that the most effective method of subsampling is to collect pollen separately from each male parent. In following this practice each sample must be used once to provide a fresh control, a condition that adds to the field work and subsequent classification. However, experience with composite samples representing the output of many plants has proved so unsatisfactory and of such doubtful reliability that for the last few years all pollinations in these investigations have been made on an individual plant basis.

This method does not wholly eliminate sampling difficulties, as the individual plant collection must be subdivided for various treatments; however, it is the best method possible under the conditions. A certain portion of the discordant results encountered throughout these investigations and the large errors so frequently involved in the populations of ears may properly be ascribed to difficulties in sampling masses of pollen.

COMPARISONS BETWEEN WAXY AND HORNY POLLEN GRAINS

SIZE

Two methods have been used to determine whether waxy pollen grains differ in size from horny pollen grains. In one method the stained grains were measured under the microscope by means of an eyepiece micrometer, but since there is the possibility that the two forms of starch may expand unequally in the staining medium, thus giving spurious results, an effort was made to separate the two sorts of grains by means of sieves.

A nest of sieves was made up of fine wire bolting screens having, respectively, 140, 170, 200, 220, and 270 wires to the inch. Although the best available screen cloths were procured and the most uniform areas of the material were selected for the sieves the apertures were quite variable. The mean sizes of the apertures of the sieves as given by the manufacturer were 0.105, 0.088, 0.074, 0.062, and 0.053 mm, respectively, but in experiments of this nature the aperture of maximum size in each sieve is important. The sieves are referred to throughout the text by the number of wires to the inch, which is the manufacturer's designation.

Some difficulty was found in the mechanics of sieving, since many pollen samples tended to clump if agitated for a few minutes. A few samples were obtained where the pollen sifted through the sieves almost like dry sand. As pollen grains differ greatly in size from plant to plant, possibly because of differences in moisture content, the samples used in the sieve tests were confined in each case to single male parents.

After separation, the pollen grains left upon each sieve were used to pollinate plants homozygous for the waxy gene, and in some cases samples were classified microscopically. These two methods of determining possible differences in size do not necessarily lead to the same result, for it is quite possible that differences in ability to effect fertilization might exist in the different size groups.

Table 2 shows the most complete series of ears, representing six sieve sizes, obtained from a single sample of heterozygous pollen. There is some indication that the intermediate pollen sizes (as determined by the size of the sieves) have the highest proportion of

functioning waxy pollen grains. However, the duplicate pollinations from the same sieve sizes gave rather widely different results, and the errors are such that no definite conclusion can be reached as to the certainty of this observation. It does seem clear that there is a significant deficiency of functioning waxy pollen grains in the smallest size group. Whether this deficiency indicates that the waxy grains were retained on the sieves of larger aperture or that the small waxy grains did not effect fertilization so well as the small horny grains cannot be determined from these data.

TABLE 2.—*Waxy and horny seeds obtained when pollen (single sample from plant 2632) lodging on the various sieves was used on ears homozygous for the waxy gene*

Female plant no.[1]	Pollen from screen no.	Seeds obtained			
		Total	Horny	Waxy	
		Number	*Number*	*Number*	*Percent*
305	140	428	217	211	49.3±1.6
306	140	114	61	53	46.5±3.2
303	170	427	186	241	56.4±1.6
304	170	334	164	170	50.9±1.8
297	200	622	265	357	57.4±1.3
295	220	162	83	79	48.8±2.7
294	270	440	222	218	49.5±1.6
293	(2)	59	37	22	37.3±3.5
Total		2,586	1,235	1,351	52.24±.66

[1] 305–306 and 303–304 duplicate pollinations; χ^2 between the combined 305 and 306 and the combined 303 and 304=3.56 (P=0.057); χ^2 between plant 293 and the combined 303 and 304=6.135 (P=0.013).
[2] Pollen that passed through all sieves.

TABLE 3.—*Analysis of pollen ratios and sizes in samples of sieved pollen from individual plants*

Source of pollen and treatment	Seeds from pollinations		Difference between seeds and pollen		Pollen classified microscopically						Red pollen—blue pollen	
							Blue-staining		Red-staining		Difference in size	S. E.
	Waxy[1]	S. E.	Waxy	S. E.	Waxy	S. E.	Size	S. E.	Size	S. E.		
Plant 7133:	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*
Unsieved	50.3	3.1					0.08512	0.00060	0.08496	0.00049	−0.00016	0.00078
Caught on—												
Sieve 140	53.1	2.2					.08264	.00049	.08312	.00057	.00048	.00074
Sieve 200	53.4	2.2					.08384	.00045	.08344	.00045	−.00040	.00064
Sieve 270	52.3	2.2					.08280	.00049	.08400	.00053	.00120	.00072
Bottom	52.6	2.2					.08200	.00053	.08224	.00057	.00024	.00078
Plant 7505:												
Unsieved	51.4	2.1	0.0	3.04	51.4	2.2	.09840	.00072	.09392	.00064	−.00448	.00096
Caught on—												
Sieve 140	44.4	11.7	5.3	11.90	49.7	2.2	.09248	.00052	.09296	.00050	.00048	.00072
Sieve 170	44.0	1.3	4.3	2.55	48.3	2.2	.09200	.00041	.09216	.00048	.00016	.00064
Sieve 200	43.8	2.5	5.8	3.32	49.6	2.2	.08968	.00044	.08944	.00050	−.00024	.00066
Plant 3425:												
Caught on—												
Sieve 170	56.0	1.3					.10144	.00161	.10176	.00096	.00032	.00194
Sieve 200	41.5	1.3					.08968	.00098	.08872	.00060	−.00096	.00112
Plant 7256:												
Caught on—												
Sieve 140							.07872	.00060	.07992	.00092	.00120	.00110
Sieve 170							.07856	.00066	.07772	.00056	−.00084	.00086

[1] Percentage of waxy seeds obtained when the pollen lodging on the sieve indicated was used on ears homozygous for the waxy gene.

No other pollen sample provided so many sizes; the majority of the samples lodged on only two or three sieves.

Waxy-horny ratios, together with micrometer measurements of the pollen grains, were determined for three samples of sieved pollen that was classified microscopically. The most complete series is that for the pollen sample from plant 7133, the data for which are given in table 3.

In this sample there is no evidence that the waxy pollen grains differ in size from the horny grains or that the sieves were at all effective in sorting the two kinds of grains.

As a check on the effect of the staining fluid on the size of grains, 50 grains were measured dry from the sample left on sieve 200. These grains, of course, could not be classified into waxy and horny, so the comparison must be made with the mean size of the stained grains from the same sieve. The mean size of the dry grains was found to be 0.08912 ± 0.00056 mm, whereas the mean size of the stained grains from this same sieve was 0.08364 ± 0.00044 mm. The difference (0.00548 ± 0.00071) is clearly significant but is in the direction of a shrinkage of the stained grains.

In the second of these samples the pollen from each sieve was subdivided, one lot being classified and measured under the microscope, the other being used to pollinate homozygous waxy ears. The data are shown in table 3.

The classifications made under the microscope show that all samples closely approximate 50 percent waxy, the widest departure being found on sieve 170 where the percentage (48.3) departs from 50 with χ^2 only 0.5 ($P=0.479$). In the unsieved pollen the blue-staining grains were 4.8 percent larger than the red-staining grains—a difference that is 4.75 times its standard error, giving $P=0.000002$. However, there is some evidence that the unsieved pollen was not a random sample of the sieved pollen, for the mean size of the unsieved grains was 0.09616 ± 0.00053 mm, whereas the mean size of the pollen grains stopped by the sieve with the largest apertures (140) was 0.09272 ± 0.00035 mm. The difference (0.00344 ± 0.00063) is more than 5 times its standard error. No explanation can be offered for this discrepancy in size.

When the ears resulting from the use of these several samples of pollen are examined an entirely different situation is encountered. The percentage of waxy seeds obtained from unsieved pollen is exactly the same as the percentage of waxy pollen found in counting the pollen grains under the microscope. The exactness of this agreement is, of course, merely a coincidence. Only 18 seeds were obtained from the pollen used from sieve 140, and the percentage is included in the table simply for completeness. The percentages of waxy in the other two samples are both significantly lower than that found in the unsieved pollen, indicating that, even though the actual proportion of waxy to horny pollen grains remains the same in all size groups, in the small sizes waxy grains are less effective than horny grains in accomplishing fertilization.

There are no significant differences between the percentages of waxy as determined by using the pollen to produce seeds and as determined by microscopic examination.

The third sieved sample (table 3) provided only two sizes, the pollen all lodging on sieves 170 and 200. The difference in the per-

centage of waxy pollen grains (14.5 ± 1.84) is clearly significant, the pollen passing to sieve 200 having the lower percentage of waxy grains. However, within the sieved samples there was no significant difference in size between the two sorts of grains.

One other sample of pollen was sieved and the sizes of grains were measured (table 3), but through inadvertence no record was made of the proportion of waxy to horny pollen grains.

This sample serves to emphasize the fact that within a sieve class the two sorts of pollen grains are of the same size. With this sample, to provide a check on possible distortion of size by the staining medium, dry pollen was measured. That from sieve 140 had a mean size of 0.07364 ± 0.0078 mm and that from sieve 170 had a mean size of 0.07612 ± 0.0006 mm. The mean size of the dry grains on sieve 170 exceeded that of the dry grains on sieve 140 by an amount that was 2.3 times its standard error. This relationship is reversed in the stained grains, the mean size of the red grains from sieve 140 exceeding that from sieve 170 by an amount that was 3.0 times its standard error. Such results indicate that despite the manufacturer's rating there is no effective difference in the sizes of the apertures in sieves 140 and 170.

In another sample, not shown in table 3, the pollen all lodged on sieves 170 and 200. That on the sieve of larger aperture proved to have by count under the microscope 53.5 ± 1.57 percent waxy grains and that on the sieve of smaller aperture 46.0 ± 0.95 percent waxy grains. The difference (7.5 ± 1.83) is over four times as large as its standard error.

In summarizing the evidence for the pollen classified under the microscope, it would appear that some plants produce pollen of which the horny grains are somewhat more likely to pass a sieve of a given size than are the waxy grains. It has been demonstrated that there is not always a corresponding difference between the sizes of the two sorts of grains. Measurements of pollen grains in unsieved samples indicate that it is questionable whether the sieves functioned as selectors of size. The observed results could be explained by slight differences in stickiness between waxy and horny grains.

From the samples used in pollinations there is strong reason to conclude that in pollen grains of the smaller sizes the waxy pollen is at a disadvantage in effecting fertilization.

Before leaving this subject it should be noted that the pollen grains from the several samples differ significantly in size. They range from 0.07 to 0.10 mm, with errors in the third decimal place.

WEIGHT

For the purpose of disclosing differences in weight between waxy and horny pollen grains, samples of pollen from heterozygous plants were separated by means of an air blast.

The apparatus consisted of a vertical glass tube about one-half inch in diameter and 30 inches long, which was bent at the upper end to deflect the floating grains into a suitable container. The lower end was closed with a fine-meshed bolting cloth and inserted into the neck of a large flask. Air under pressure was admitted to the flask by means of a side connection. The flask served to smooth convection currents and provided a more even air pressure on the bolting cloth.

The pollen to be tested was placed on the bolting cloth and with the increase in air pressure rose through the tube and spilled into the container. No attempt was made to record air pressure, which was controlled by a needle valve and increased as required to drive over the last grains.

The pollen grains that floated through the tube with the lowest pressure were kept separate from those that rose through the tube only with the highest pressure. Samples of the first and last lots were stained with iodine and counted. The bulk of the pollen was applied to silks of plants homozygous for waxy for an eventual seed count.

The two sorts of grains in both the light and heavy samples were essentially equal in number and the χ^2 of the difference between the light and the heavy weight was 0.80 ($P=0.37$).

Only four samples of pollen produced ears from both the heavy and the light weight. In the most successful of these, three ears were produced from each of the weight groups. Combining the three ears from each group, the χ^2 of the difference between the ratios of horny to waxy seeds is found to be 3.89 ($P=0.048$) indicating that the air blast did not separate the two sorts of pollen grains.

As a further analysis of these data, 10 ears resulting from heavy pollen were combined, and the mean percentage of waxy seeds was compared with that obtained when 10 ears resulting from light pollen were combined. The heavy pollen gave 48.11 ± 0.85 percent waxy and the light pollen gave 47.92 ± 0.57 percent waxy. The difference between the two groups (0.19 ± 1.02) is not significant. From these data it may be concluded that any difference in the percentage of waxy seeds due to the use of pollen of different weights could not have been greater than 3 percent.

SPECIFIC GRAVITY

As a means of determining whether waxy pollen grains differed from horny pollen grains in specific gravity, pollen from a heterozygous plant was placed in a tube filled with a solution of chloroform and alcohol in the proportion of 38 : 6. This solution could be so accurately balanced that the temperature would determine whether the grains would sink or float; a drop of 1° or 2° C. would sink all pollen grains, whereas an increase of a few degrees would bring the grains to the surface. By adjusting the proportions of chloroform and alcohol and holding the tubes under constant temperature, a fairly good separation of pollen grains could be obtained. Pollen grains from the top and bottom of the tube were pipetted off, stained with iodine, and classified under the microscope. Several individual plant samples were tried. In one the floating grains gave 69 horny to 68 waxy and the sunken grains gave 161 horny to 148 waxy. The χ^2 of the difference between the sunken and the floating grains is 0.114 ($P=0.74$), indicating a chance difference between the two sets. With another sample the floating grains were classified as 1,135 waxy to 929 horny, and the sunken grains gave 536 waxy to 494 horny.

Fifty-five percent of the floating grains and 52 percent of the sunken grains were waxy. The difference of 3 percent has a probability of 0.066. From these tests it may be concluded that the two sorts of pollen grains do not differ in specific gravity.

ELECTRICAL TEST

An attempt was made to separate the two sorts of pollen grains electrically, but this proved unsuccessful. Two strips of zinc were suspended about one-quarter of an inch apart in a tube containing chloroform and alcohol in which heterozygous pollen was suspended. These zinc poles were connected with a 6-volt storage battery, but no lateral movement of the suspended pollen grains could be detected.

EFFECT OF VARIOUS FACTORS ON RATIO OF WAXY TO HORNY SEEDS

TIME OF ANTHESIS

It has been suggested that the effects observed in stored pollen might be explained by a differential maturity of the two types of grains; that a simple differential death rate would account for percentages of waxy seed greater than 50 in stored pollen but would not explain percentages less than 50 when fresh pollen is used.

If waxy pollen grains are in fact immature at anthesis, then the very first pollen shed in the morning should result in ears with the lowest percentage of waxy seeds, and this percentage should increase with time. To test this point an effort was made to collect pollen early in the morning, but on most August days in the vicinity of Washington heavy dews delay pollen shedding until 8 a. m. Even when a few favorably situated plants shed some pollen before this hour, the moisture that accumulated on the spikelets interfered with collection. However, one series beginning at 6:45 a. m. was successful. From this series there is no evidence that the waxy pollen grains are less mature when shed early in the morning than when shed later. The data are shown in table 4.

TABLE 4.—*Effect of hour of collection of pollen on percentage of waxy seeds*

Hour of collection	Ears	Total seeds	Waxy seeds
A. m.	*Number*	*Number*	*Percent*
6:45	6	1,317	46.6±1.1
7:15	5	1,554	50.3± .5
7:55	7	2,290	46.7± .9
8:30	15	6,298	45.5± .8
Mean	33	11,459	46.5± .5

TABLE 5.—*Effect of hour of collection of pollen from each of 4 plants on percentage of waxy seeds resulting from fresh applications, July 30, 1932*

Source of pollen (plant no.)	Waxy seeds from pollen collected at indicated hour		
	8 a. m.	11:30 a. m.	Difference (11:30–8 a. m.)
	Percent	*Percent*	*Percent*
1	47.3±0.95	51.1±1.12	3.8±1.5
2	45.7±1.03	44.7±1.13	−1.0±1.5
3	47.9± .97	42.9±1.02	−5.0±1.4
4	43.8± .90	45.8±1.09	2.0±1.4

With the exception of the collection made at 7:15 a. m., the ratios of waxy to horny seeds are significantly less than equality but do not differ from one another. In one other case pollen was collected from four plants at 8 a. m. and was applied immediately. A second collection of pollen was made from these same plants 3½ hours later. The results are shown in table 5. Of the four differences, one is three and one-half times its error; but since it is one difference in a series of four, the odds against its being due to chance are about 16 to 1.

The time of day at which the pollen is shed, therefore, seems to bear no relation to the deficiency of waxy seeds resulting from the use of fresh pollen. This deficiency may be due to immaturity, but if so the waxy grains are equally immature irrespective of the time of anthesis. The results accord better with the observations of Sprague (*21*), i. e., that waxy pollen grains germinate more slowly than horny grains.

CONDITIONS OF POLLEN STORAGE

Storage in Sunlight

Although in 1925, 1926, 1927, and 1929 pollen exposed for as long as 7 hours to sunlight was capable of functioning in fertilization, similar exposures in 1928 were uniformly lethal, and much shorter periods tried in 1930 and 1931 (some as short as 10 minutes) were practically lethal, only a few seeds being obtained. In 1932 a few exposures for as long as 5 hours were successful. Exposures for shorter periods (15 to 45 minutes) showed no lethal effects. It became possible in 1932 to measure the degree of illumination by means of photronic cells, and it was found that the brightest cloudless summer days in the vicinity of Washington, D. C., registered 12,500 foot-candles in direct sunlight and that the maximum observed in Arizona was 13,000 foot-candles. Under a single layer of glassine paper the radiation was reduced about one-third.

On most days in late July and early August, when these experiments were conducted, the sky is far from cloudless and the illumination varies rapidly from 2,000 to 12,000 foot-candles. Since the experiments were made over a period of from 10 days to 2 weeks in any one year, it is not believed that the lethal effects obtained in the years 1928, 1930, and 1931 were caused by excessive radiation.

While no measurements of radiation were made previous to 1932, records were kept as to whether the day was clear, partly cloudy, or cloudy. So far as these records serve, they fail to support the idea that the death of pollen in 1928, 1930, and 1931 was due to unusually intense radiation. It is more reasonable to assume that in these years pollen was more sensitive to radiation than in the other years. This hypothesis receives support from the fact that pollen from different plants does not react alike to storage treatments.

With a knowledge of these limitations in mind, the results obtained from exposures of pollen to sunlight may be examined.

In 1927 no pollinations were made with fresh pollen, and the comparison for this year is limited to sun storage as contrasted with storage in the dark for the same period of time. The comparisons are all on an individual plant basis, each plant furnishing pollen for a sun and for a dark exposure. All the samples were in glassine bags, and the sun exposures were made on a flat table in direct sunlight. The time intervals ranged from 7½ to 7¾ hours.

Only 13 pairs of pollinations from light-dark treatments were successful. In 12 of the 13 cases the pollen exposed to direct sunlight produced ears with a higher percentage of waxy seeds than the duplicate samples stored during the same period in the dark. The mean excess for the series was 20.13 ± 2.01 percent, despite the fact that the dark series averaged 50.4 ± 0.7. The mean difference and its error is weighted by $1/E_d^2$; the details of this statistical procedure have been published elsewhere (*8*).

As part of the series of heat treatments given at Arlington, Va., in 1928, a series of duplicates was exposed to sunlight. Most of these were for periods of 5½ hours and all proved to be lethal. The only pollen that produced seeds after exposure to sunlight was a composite sample spread in a thin layer in a large uncovered Petri dish resting on a block of ice. Seven ears were obtained from this treatment, which lasted for 4½ hours. The mean percentage of waxy seeds was 47.30 ± 0.73. No other treatments given on this day produced ears, so there is no other population with which to compare this percentage. Since, however, the average percentage of waxy seeds from fresh pollinations made at Arlington in this year with composite samples of pollen was 46.24 ± 0.78, there is every reason to conclude that the sample stored on ice in direct sunlight for 4½ hours was not affected by the exposure.

In 1929, at Lanham, Md., the length of the period of exposure to sunlight ranged from 6 hours and 40 minutes to 8 hours and 45 minutes, but the individual variability was so great that no conclusions can be drawn as to the relationship of time to the degree of alteration in the waxy-horny ratio.

All pollinations were made on the individual plant basis, and the comparisons are between pollen that was used fresh and pollen that was stored.

The pollinations were made on 2 days, August 5 and 8. August 5 was a bright clear cold day, with radiation probably approximating 12,000 foot-candles, whereas August 8 was cloudy. Only three pairs of pollinations were successful on August 5, all showing a pronounced effect of the exposure to sunlight. The mean difference in the percentage of waxy seeds from stored pollen and that from fresh pollen was 16.4 ± 3.3, the stored pollen producing the higher percentage

The pollinations made on August 8 were more successful, doubtless because of the less rigorous conditions prevailing during exposure. The effect of the treatment was far less than that of 3 days earlier, though the difference in the percentage of waxy seeds obtained from stored pollen and that obtained from fresh pollen was 4.9 ± 1.3 percent, in favor of the stored pollen.

Although fresh pollen produced ears with percentages of waxy seeds ranging from 33.1 to 55.9, there was no tendency for these differences to be maintained when stored pollen was used. The correlation between the percentage of waxy seeds resulting from the use of fresh pollen and that obtained from stored pollen was only 0.16 ± 0.13, indicating that the conditions of storage obliterated any differences between fresh samples.

If the storage operated to bring the functioning waxy grains up to equality with the horny grains, a negative correlation should be found between the percentage of waxy seeds on the ears resulting from fresh pollen and the difference between this percentage and that obtained

from the use of stored pollen. This would follow since the fresh samples giving the lowest percentage should be (under the premise) the samples that produced the largest differences. The correlation is found to be -0.302, and although the coefficient has no statistical significance, P lying between 0.1 and 0.2, it does indicate that the deficient percentages obtained from fresh pollen tend to be corrected in the stored pollen.

At Arlington, on August 8, a series of exposures to sunlight was made covering a period of 4 hours. The strains used were identical with those grown at Lanham. Only nine pairs of ears were obtained, and these showed no evidence of alteration in the proportion of waxy to horny pollen grains when exposed to sunlight. None of the 10 differences is statistically significant.

This result may be compared with the results obtained at Lanham on the same day, where 13 of the 26 differences were significant, 10 being in the direction of a higher percentage of waxy seeds from treated pollen. Since the exposure at Arlington was for 4 hours and the shortest exposure at Lanham was 6 hours and 50 minutes, the lack of agreement between the two places may be due to this difference in period of exposure.

On the same day at Arlington a second group of pollinations was made, in which the fresh pollen was used on homozygous waxy plants and the stored pollen was used to self-fertilize the plants that functioned as male parents. This was done because there was a possibility that the pollen might behave differently when used on the stigma of the plant that produced it than when used on sister plants. The fresh pollen proved to have 47.12 ± 0.57 percent waxy grains. The expected percentage of waxy seeds when this pollen is used on plants heterozygous for waxy is 23.6. The self-pollinated ears resulting from the use of stored pollen had 23.5 ± 0.4 percent of the seeds waxy. This agreement with the percentage expected from the known proportion of waxy to horny pollen grains in the fresh pollen is added evidence that 4 hours' exposure to sunlight failed to alter the relative effectiveness of waxy pollen grains.

In nine instances stored pollen was used both in backcrosses and in self-pollinations, permitting direct comparisons of the two types of pollination. The mean difference between the self-pollinated and the backcrossed ears, after doubling the percentages in the groups of self-pollinated ears to make them comparable with those obtained from backcrossing, is found to be -1.4 ± 1.7, showing that the results of pollen storage are not affected by the nature of the pollination, i. e. whether selfed or backcrossed.

There was no correlation between the percentage of waxy seeds on the backcrossed and on the selfed ears where stored pollen was applied ($\rho = -0.221 \pm 0.224$). With only nine comparisons, however, the correlation must be very close for statistical significance.

At Arlington, Va., in 1929, an attempt was made to accumulate the data necessary to determine the change in proportion of functioning waxy pollen grains with increase in time of storage.

A bulk sample of pollen was collected, subdivided into small containers, some of these samples were used immediately for fresh controls and the remainder stored in the sun. Each hour a certain number of exposed samples were applied to ears of homozygous plants. It

was found necessary to make two collections of pollen, and unfortunately these two lots did not react alike to storage.

The summarized data are shown in table 6. As will be seen, the pollen from the first lot showed a change in the percentage of waxy seeds after 2 hours' exposure to sunlight and an increased alteration with 3 hours of storage, which was the final period for the first bulk sample of pollen. The second lot, although showing an increase in percentage of waxy with the passage of time up to 6 hours, fell far short of the change observed in 3 hours in the first lot. Since both lots of pollen had identical exposures for the first 3 hours, the discrepancy between them cannot be charged to different amounts of radiation. Both lots of pollen were alike in their behavior when used fresh, and since they consisted of bulk samples from sister plants there can be no question of plant sensitivity. Further, since the pollen was all subdivided into small lots, each subsample containing enough pollen for two ears, it hardly can be maintained that variation in the quantity of pollen was a factor. Although it is probably true that the samples contained unequal amounts of pollen, it is unlikely that all the subsamples of one collection contained more or less pollen than all those of the other collection.

TABLE 6.—*Percentage of waxy seeds obtained from fresh and from sun-treated pollen, Arlington, Va., August 6, 1929*

Treatment of pollen	Ears	Total seeds	Waxy seeds	Treatment of pollen	Ears	Total seeds	Waxy seeds
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
Fresh (first collection)	6	2,473	45.8±1.4	Fresh (second collection)	11	4,775	43.7± .9
Hours in sun:				Hours in sun:			
1	13	5,258	45.5± .7	4	14	6,479	44.1± .8
2	10	3,223	51.6±1.7	5	10	5,143	47.0±1.0
3	11	3,196	63.0±1.4	6	12	4,142	52.7±1.4

At Sacaton, Ariz., in 1929, two lots of ears were self-pollinated, one lot with pollen collected and applied between 7:30 and 8 a. m., the other with pollen collected during this period and exposed to direct sunlight through one thickness of glassine paper for 8 hours and 45 minutes. Since the ears were self-pollinated, identical plants are not involved, but the number of plants was such that an average sample was assured. The ears obtained from the use of fresh pollen had a mean of 20.9 ± 0.5 percent waxy seeds and those resulting from the use of stored pollen had 23.0 ± 1.1 percent waxy. The difference between the two groups is not significant. There is a significant negative correlation between the number of seeds and the percentage of waxy in the group of ears obtained from the use of stored pollen, ρ equaling -0.584, and P being less than 0.01. In the fresh pollinations the correlation coefficient is of the same sign but is not significant ($\rho = -0.291$).

There is no difference between the two populations in mean seed number, the stored group having a mean of 157 and the fresh group a mean of 150 seeds per ear. The fact that a higher percentage of waxy seeds is found when the number of seeds is small might be taken as an indication that the slower rate of germination of waxy pollen grains accounted for the observed correlation in the group of ears from stored pollen. However, this relationship would be expected

as well in the group of ears from fresh pollen, on the premise that scant quantities of pollen offer no opportunity for competition between the slow- and fast-germinating sorts of pollen grains.

The fact that no such relationship between number of seeds and percentage of waxy is found in the fresh group as is revealed in the stored group indicates that the unfavorable conditions of storage in sunlight operate to destroy horny pollen grains more rapidly than waxy grains.

At Lanham in 1932 a series of exposures was made in direct sunlight for a period of one-half hour. Half-hour exposures were made on three different days, the radiation as measured with the photronic cell ranging from 2,000 to 12,500 foot-candles. On none of the 3 days was the treatment effective, and the mean change for the combined series was an increase from treated pollen of 1.08 ± 0.41 percent waxy.

On one of the days, treatments of 15, 30, and 45 minutes were given. Several of these were successful in all categories. The data are presented in table 7. In only one instance (sample 3005, exposed for 45 minutes) was there evidence of a significant change in the proportion of functioning waxy pollen grains. This sample shows a difference of 16.0 ± 2.2 percent between the effectiveness of the stored pollen and that of the fresh pollen, the stored pollen producing the higher percentage of waxy seeds.

Longer exposures were tried in this year, but only three pairs were successful. These were given 5 hours at rather low candlepower (180 to 7,000 foot-candles), with temperatures ranging from 28° to 41° C. All three samples show very great differences in the percentage of waxy seeds between ears resulting from the use of stored pollen and those from the use of pollen exposed to sunlight. The mean difference in percentage of waxy seeds for these three ears is 42.9 ± 3.3, the pollen stored in sunlight giving the highest percentage.

TABLE 7.—*Percentage of waxy seeds obtained from fresh and from sun-treated pollen* [1]

Pollen sample no.	Seeds resulting from pollen used after indicated treatment							
	Fresh		In sun 15 minutes		In sun 30 minutes		In sun 45 minutes	
	Total	Waxy	Total	Waxy	Total	Waxy	Total	Waxy
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
2437	1,347	38.6±1.1	1,223	40.2±0.9	999	45.2±1.1	676	47.9±1.3
2486	2,318	44.6± .7	1,378	48.3± .9	892	51.0±1.1	555	48.3±1.4
2816	1,962	45.3± .8	942	41.5±1.1	942	43.9±1.1	598	47.5±1.3
2825	1,418	45.3± .9	1,303	46.9± .9	936	43.6±1.1	1,278	47.2± .9
3005	2,296	45.6± .7	1,245	42.5± .9	945	48.8±1.1	250	61.6±2.1
3007	2,169	48.9± .7	1,285	45.6± .9	1,240	44.3±1.0	885	48.0±1.1
3105	582	44.7±1.4	1,100	45.9±1.0	767	47.2±1.2	7	85.7± .9

[1] The illumination varied from 4,400 to 6,400 foot-candles, and the temperature ranged from 37° to 50° C.

STORAGE AT VARIOUS TEMPERATURES AND IN OXYGEN

In order to differentiate further between temperature and light it would be desirable to have light exposures at low temperatures and heat exposures in the dark. The latter, of course, are easy to provide, but the former can be obtained only imperfectly. It is not possible to vary the illumination without a concomitant alteration

in the temperature, except by means of quartz cells and flowing water. Even with this device, which restricts the number of pollen samples handled and increases the time interval for equal energy intake, the quality of radiation is somewhat altered. Not only is it difficult to provide light exposures without heat but it is equally difficult to determine the temperature of pollen grains exposed to light. Just what proportion of the radiant energy is transformed into heat when absorbed by the opaque yellow pollen grains and how much of this heat is counteracted by evaporation it is now impossible to measure. Crude attempts have been made to estimate pollen temperatures by means of pollen-covered mercury thermometers, but the information derived from this method is admittedly inaccurate.

The tests show that such thermometers exposed to sunlight may exceed air temperatures by as much as 25° C., the amount depending on the brightness of the day, air movement, and air temperature, to say nothing of the thickness of the pollen coating and its moisture content.

If the observed increase in proportion of waxy to horny seeds following the use of pollen stored in direct sunlight is simply the result of high temperatures, then pollen stored at temperatures of 35° to 45° C. in the dark should produce similar changes in the percentage of waxy seeds.

To test this point a vertical oven was constructed with a light bulb at the bottom as the source of heat. This oven, with four wire shelves and a damper control, proved to give very constant temperatures. The four stages provided temperatures of 31°, 32°, 35°, and 45° C., which could be held within a range of 1° with infrequent attention to the damper.

Pollen collected from 50 heterozygous plants was sieved to remove anthers and thoroughly mixed. This sample was then divided into subsamples, which were placed in small glassine bags, each subsample having enough pollen to serve 2 years. The glassine bags were then numbered, and the pollen from one was used immediately to pollinate two ears of homozygous waxy plants. The silks of these ears had been cut back 24 hours previously to provide a uniform brush about 1 inch long. The bags of pollen to be treated were subjected to the four oven temperatures for 3½ hours, after which the pollen was applied to ears in the same progeny as the ears pollinated with the fresh pollen. Because of the distance from the field to the laboratory and the time consumed in making the pollinations, the period elapsing between the time the treated pollen was collected and the final application was 5½ hours. Each of the subsamples was used on 2 ears, except that in a few cases, where one of the ears had but few silks, three ears were pollinated from a single subsample. The results are summarized in table 8.

It will be seen at once that the treatments, far from increasing the percentage of waxy seeds, actually reduced it. The control lot deviated slightly but not significantly below equality, but without exception all the heat treatments tried in this experiment resulted in significant reductions in the percentage of waxy seeds.

TABLE 8.—*Percentage of waxy seeds obtained from pollen subjected to various temperatures for 3½ hours*

Treatment (° C.)	Ears	Total seeds	Waxy seeds
	Number	*Number*	*Percent*
Control [1]	3	846	47.87±1.15
31	6	2,310	39.44±1.05
32	8	1,453	38.13±1.17
35	11	3,076	40.08± .85
45	9	2,469	39.69±1.57

[1] Pollen applied immediately after subsampling.

A second experiment was conducted with pollen handled in the same fashion. In this experiment pollen was collected from 50 plants and thoroughly mixed. Subsamples were then poured into small glassine bags, which were assigned consecutive numbers. These subsamples were divided into three groups. One group was stored for 2½ hours at 37° C., another was stored for the same length of time at 10°, and the third was held in an atmosphere of oxygen drawn into jars through sulphuric acid. All three treatments gave very low percentages of waxy seeds, the mean percentages being 32.49±0.49, 31.18±0.53, and 37.73±0.45, respectively. The results of the two temperature treatments were nearly alike in the percentage of waxy seeds, indicating complete absence of temperature effect and pointing clearly to some other disturbing factor.

These ears are the first in which a reduction in the percentage of waxy seed was observed to follow the use of stored pollen, but other lots were encountered subsequently and similar reductions have been reported by Brink (*2*). Further it should be noted that all pollen produced at the Arlington Experiment Farm in 1928 and subjected to various treatments gave similar reductions in the percentage of waxy seeds. Although the experiment affords no proof that high temperatures offer the explanation for the observed effect of storage of pollen in the sunlight, it must be borne in mind that the technique followed in handling the pollen samples differed radically from that previously employed. In fact the general reductions in the percentage of waxy seeds observed in all pollen samples handled in bulk at Arlington raises the question as to whether the results are not directly ascribable to this method of handling the pollen.

In addition to the oxygen series already discussed, storage in oxygen was tried on 3 other days. As it happens, no other ears are available for comparison on 2 of these days, as all other treatments tried, such as high temperatures, carbon dioxide, etc., proved lethal. However, a series of 10 ears resulted from fresh pollen used on 1 day (Aug. 4). These 10 ears had a mean of 46.24±0.78 percent waxy seeds. The summarized data for the oxygen treatments are given in table 9.

All four groups of ears resulting from pollen stored in oxygen had less than half of the seeds waxy, and the deficiency became greater as the season advanced. No significance is attached to this relationship, as numerous attempts to discover a seasonal trend in the waxy-horny ratio in many progenies have always resulted in failure. However, Chao (*4*), working with the glutinous gene in rice (which

corresponds in effect very closely with the waxy gene in maize), found evidence of a seasonal trend in the survival of pollen grains bearing this gene.

TABLE 9.—*Percentage of waxy seeds obtained from pollen stored in oxygen for 4½ hours*

Date	Ears	Total seeds	Waxy seeds
	Number	*Number*	*Percent*
July 31	5	1,727	48.52±1.23
Aug. 1	7	2,345	46.31±1.31
Aug. 4	4	137	40.88±1.30
Aug. 7	22	9,449	37.73± .45

When the mean percentage of waxy seeds obtained from the use of fresh pollen (46.24±0.78) was compared with that obtained from pollen stored in oxygen (40.88±1.30) on August 4, the difference was found to be 5.36±1.52, the oxygen-treated pollen giving the lower percentage.

At Lanham, Md., in 1928, bulk samples of pollen were collected from heterozygous plants, but, instead of combining the pollen of 50 plants, only 10 plants were used and several collections were made. Numerous exposures to sunlight were tried, but in every case this treatment killed all the pollen.

One experiment involved dividing the bulk pollen into three subsamples, applying one subsample fresh to several ears, exposing a similar quantity to direct sunlight for several hours, and placing the third lot in an oven. Five bulk samples, collected at five different hours in 1 day, were treated in this manner. Not a single lot exposed to sunlight produced seed, but the oven samples were more successful. These are compared with the controls in table 10. The longest period of treatment corresponds with the earliest time of collection, since all treated samples were removed from the oven at the same time.

TABLE 10.—*Percentage of waxy seeds obtained from fresh pollen and from duplicate pollen samples held in the dark for various periods and at various temperatures*

Sample no.	Hour of collection	Treatment		Ears	Total seeds	Waxy seeds
		Temperature	Duration			
	A. m.	*° C.*	*Hours*	*Number*	*Number*	*Percent*
1	8:00	(1)		7	1,729	50.64±1.03
		35	8½	5	145	71.04±3.49
2	8:30	(1)		5	1,398	48.64±1.51
		26	8	5	700	46.15±1.41
		28	8	6	1,076	46.40± .82
3	9:30	(1)		8	1,980	49.04±1.06
		28	7	8	2,638	47.42±1.01
4	10:15	(1)		10	3,105	49.08± .80
		26	6¼	13	5,491	48.42± .37
5	10:50	(1)		1	168	53.00±2.50
		35	5¾	4	1,525	49.51± .95

[1] Pollen applied fresh.

It will be noted that, with the exception of the 8½-hour treatment at 35° C., all treatments resulted in a lower percentage of waxy seeds than their corresponding controls. The group of five ears resulting from the 8½-hour treatment and having a mean percentage of 71.0±3.5 waxy seeds was consistent, although, as the total (145) shows, the ears had very few seeds. So far as known, there was no difference in these samples other than the time at which the pollen was collected and the length of the storage period. However, from the fact that the pollen collected at 10:50 a. m. and stored for 5¾ hours at 35° gave no evidence of an increased survival of waxy pollen grains, it is highly doubtful that the change observed in lot 1 after 2¾ more hours of storage could have resulted from the additional time unless a definite threshold reaction be assumed. It seems more likely that the pollen collected at the earlier period was in some manner more sensitive to the conditions imposed and that the waxy grains were slightly more able to resist the unfavorable treatment. Certainly the number of seeds obtained from this lot of treated pollen was very much less than that obtained from lot 5.

In the years 1931 and 1932 all pollinations were made on the individual plant basis. Each sample of pollen from a heterozygous plant was subdivided and one lot was used fresh as a control. Various periods of exposure and several stages of heat treatment were tried. The results are summarized in table 11. When the array of mean differences is examined, it is seen that the percentages of waxy seeds from stored pollen range from a deficiency of 3.0±0.9 to an excess of 17.3±4.6. The duration of the treatments ranged from 15 minutes to 2 hours, and the temperature to which the several samples were subjected ranged from 40° to 50° C. In attempting to derive order from this series, it became apparent that the closest relationship is that between the length of the exposure period and the degree of alteration in the proportion of functioning waxy pollen. The coefficients of correlation of ranks calculated for these variables are as follows: Between the difference in the percentage of waxy seeds and duration of exposure $\rho=0.614$; between the difference and temperature $\rho=-0.25$; and between duration and temperature $\rho=-0.7$. The last-named coefficient, of course, results largely from design, since for the most part the pollen samples were kept at the highest temperatures for the shorter periods. With only 9 points, these coefficients can be only suggestive.

There can be no question but that the series of ears resulting from the use of pollen stored in the dark for 2 hours at 41° C. in 1932 had significantly higher percentages of waxy seeds than the ears pollinated with duplicate samples of fresh pollen. However, even in this series of nine comparisons there were three where no effect of storage was obtained. In the other groupings there are scattered samples where unquestionable alterations have occurred, especially in the series of 12 ears held on July 30, 1932, for 1½ hours at 47°. The series as a whole is so variable that the mean change is hardly significant.

In addition to the scattered samples showing increased effectiveness of waxy pollen grains following storage, there are several cases where the treatment seems to have acted unfavorably on the waxy pollen.

TABLE 11.—*Percentage of waxy seeds from fresh pollen and from pollen stored for various periods and at various temperatures* [1]

Date	Treatment		Paired ears	Total seeds from indicated pollen		Difference in waxy seeds from fresh and from stored pollen
	Duration	Temperature		Fresh	Stored	
1931	*Minutes*	*° C.*	*Number*	*Number*	*Number*	*Percent*
July 28	15	42	9	6,316	5,049	3.0±0.9
July 29	30	49	5	2,261	1,316	−3.6±3.0
July 30	30	44	4	2,425	1,270	.7±2.2
Aug. 1	30	40	2	1,121	1,857	1.4±1.0
Aug. 2	20	50	7	3,999	4,062	1.1±2.3
1932						
July 28	90	44	2	1,969	2,128	1.1±2.1
July 30	90	47	12	16,613	13,724	−7.9±2.6
Aug. 3	75	41	8	10,450	10,031	−.9± .7
Do	120	41	9	11,660	8,184	−17.3±4.6

[1] Pollinations were made with collections from individual plants, each pollen parent furnishing a fresh and a treated sample.

One other series of comparisons of pollen treated in the dark remains to be discussed. In this last series individual plant samples were subdivided into two lots. One lot was stored at 10° C., the other at 25° for periods of 7 hours and 10 minutes to 7 hours and 30 minutes, both being in the dark. The pollen stored at 25° gave a slighly higher percentage of waxy seeds than that stored at 10° but the difference ($2.6 \pm .9$) is not significant. Both samples gave mean percentages of waxy seeds slightly above 50, which is unusual.

STORAGE UNDER ARTIFICIAL LIGHT

For experiments designed to determine the effect of light on the survival of pollen grains, the sun constitutes a capricious and an uncertain source of illumination since sunlight varies in intensity with each passing cloud. Even on bright clear days the intensity of radiation varies, and exposures to uniform amounts of radiation are not often obtainable. The chief advantages of sunlight lie in the amount of energy delivered and in the area covered, which makes possible the exposure of large numbers of samples. These advantages are offset by the difficulty in estimating the amount of radiation delivered over a given period. The development of the photronic cell has provided a means of rapidly measuring changes in radiation, but where the radiation is varying during an exposure period even frequent readings of this instrument permit only a coarse estimate of the amount of radiation. Accordingly a controlled source of illumination was sought.

It was known from the experiments of Brink (*2*) with ultraviolet and also from the fact that sunlight is effective through glassine paper (having a transmission spectrum similar to that of window glass) that wave lengths in the ultraviolet region were not essential in differentiating waxy from horny pollen grains.

It was desirable that the artificial source of illumination should have an emission spectrum similar to sunlight and that the energy output should be such that long exposures were not required.

Providentially there was introduced at about this time a lamp designed to meet the therapeutic fad of the moment for home suntanning. This lamp ingeniously combines the mercury vapor arc with a glowing tungsten filament and when equipped with a Corex glass bulb affords in simple form a fair approximation to sunlight, though somewhat deficient in the middle region of the spectrum. The radiant energy at a distance of 20 cm from the source is roughly 0.1 that of sunlight at the earth's surface on a bright day.

This light was placed in operation in 1930, a year that in the vicinity of Washington, D. C., was notable for an extended drought. Preliminary experiments showed that pollen exposed to this light for one-half hour failed to germinate on corn silks, but shorter exposures offered more hope. The treatments were planned, therefore, on the basis of exposures of 5, 10, and 15 minutes, and in addition some samples were illuminated through a quartz cell carrying a stream of cold water as a means of preventing a rise in temperature.[4]

The rigors of the season played such havoc with the results that few ears were obtained and these had very few seeds each. A single complete set, having enough seeds to carry some statistical weight, represents the results for this year. These data are shown in table 12.

TABLE 12.—*Percentage of waxy seeds obtained from subsamples of pollen from a single plant, applied fresh or after exposure for various periods to artificial light of 1,200 foot-candles* [1]

Treatment		Total seeds	Waxy seeds
Duration	Temperature		
Minutes	*°C.*	*Number*	*Percent*
(2)	36	292	48.6±2.0
5	38	122	54.9±3.0
10	40	100	64.0±3.2
15	49	177	71.8±2.3
15 [3]	43	135	51.1±2.9

[1] Estimated from known distance between lamp and pollen.
[2] Pollen applied fresh.
[3] Pollen protected from high temperature by quartz water cell.

The pollen used on these five ears was from a single heterozygous plant, and the results show that the factors operating during the exposures to light, in the absence of the quartz water cell, brought about a decided alteration in the proportion of waxy to horny pollen grains functioning in fertilization. The single ear resulting from the use of pollen exposed to light for 15 minutes but protected from high temperature by the quartz cell shows equally clearly that light alone does not affect these two sorts of pollen grains differentially.

The temperature as measured by an exposed mercury-bulb thermometer rose under the quartz water cell from 36° to 43° C., whereas without the protection of the water cell the temperature attained a maximum of 49°. As it has been shown that temperatures of this degree for such short periods are ineffective, the possibility may be entertained that the differential functioning of these two sorts of pollen grains is brought about by the combination of light and heat.

[4] The writer is indebted to Dr. F. S. Brackett, formerly of the Division of Radiation and Organisms, Smithsonian Institution, for suggesting the use of this device and for the cell itself.

Slight support for this hypothesis is derived from a summary of all the seeds obtained from these experiments in 1930.

The high mortality of all pollen in 1930 prevented comparisons of individual pollen samples, but the seeds may be summarized. From the use of fresh pollen, 3,862 seeds were obtained having 50.0 ± 0.6 percent waxy; from pollen treated with artificial light not protected by the quartz water cell, 1,094 seeds were obtained having 53.0 ± 1.0 percent waxy; when the pollen was screened by the quartz water cell, 2,479 seeds were obtained with 49.1 ± 0.7 percent waxy; and when pollen was stored in the dark at temperatures identical with those of the unprotected light series, 260 seeds were obtained having 50.1 ± 2.1 percent waxy. The errors in the above summary are calculated from the total number of seeds and no great significance can be attached to the results. There is thus a slight indication that light plus heat produces an effect not obtained with either factor alone, but the data are far from conclusive.

Since the data obtained in 1930 were inadequate, an extensive series of exposures to artificial light was tried in 1931. These exposures ranged from 5 to 45 minutes, all without the quartz water cell.[5] The results are shown in the first 11 entries in table 13. Not one of the treatments produced a significant alteration in the proportion of waxy to horny pollen functioning in fertilization.

TABLE 13.—*Comparison of results obtained from fresh pollen and from pollen exposed for various periods to artificial light of 1,200 foot-candles* [1]

Treatment [2]			Paired comparisons	Total seeds from indicated pollen		Difference in waxy seeds from fresh and from treated pollen
Date	Duration	Temperature		Fresh	Treated	
1931	*Minutes*	° *C.*	*Number*	*Number*	*Number*	*Percent*
July 25	20		5	2,331	2,027	-0.2 ± 2.0
July 26	10		11	3,975	3,717	$.0 \pm 1.2$
Do	5		6	2,798	2,508	1.5 ± 1.7
July 28	5	47	6	3,908	2,790	3.8 ± 1.7
Do	15	45	7	4,968	5,005	$.0 \pm 1.4$
July 30	20	44	5	2,689	2,463	-3.6 ± 2.1
Do	30	46	5	5,705	2,181	1.7 ± 1.3
Aug. 1	15	39	9	4,888	2,901	2.5 ± 1.1
Do	30	42	9	5,209	6,761	4.6 ± 1.1
Do	45	43	4	1,514	1,550	$.2 \pm 1.7$
Aug. 2	20	44	5	2,930	1,581	$-.2 \pm 2.0$
1932						
July 30	[3] 60	37	6	10,130	3,302	-9.6 ± 3.1
Do	90	47	5	7,596	25	-55.0 ± 3.0
Aug. 3	75	40	5	6,540	1,384	-47.0 ± 1.9
Do	120	41	6	8,224	157	-58.8 ± 1.2

[1] Estimated from known distance between lamp and pollen. At this distance the pollen was exposed to 1,200 foot-candles in 1932, when the illumination was measured by the photronic cell.

[2] Pollinations were made from collections from individual plants, each pollen parent furnishing a fresh and a treated sample.

[3] At 800 foot-candles.

A single experiment was arranged whereby the pollen samples were exposed at different distances from the source of light, all being exposed at the same time and for the same period. The distances were so arranged that the uppermost sample received four times the radiant

[5] The photronic cell was not available in this year, but the following year it was found that at the distance that separated them from the lamp the pollen grains were subjected to light of an intensity of 1,200 foot-candles.

energy of the lowest sample. The comparison involved fresh pollen and pollen exposed for 20 minutes at distances of 32 cm, 24 cm, and 16 cm from the light. The following percentages of waxy seeds were obtained: From the fresh pollen, 45.6 ± 0.7; from the pollen exposed at 32 cm, 43.2 ± 0.6; at 24 cm, 44.1 ± 0.7; and at 16 cm, 45.3 ± 0.8. Clearly for the time involved (20 minutes) the light produced no effect on the proportions of the two sorts of pollen grains, nor is there any evidence that the ratio varied with the energy received.

In view of the conflicting results in these experiments with pollen ratios, further experiments were conducted in 1932. The summarized results are shown in the last four entries in table 13. Exposures as short as 1 hour with an illumination of 800 foot-candles, as measured with the photronic cell, produced a marked differential in the survival of the two sorts of pollen grains in certain samples. When the light intensity was raised to 1,200 foot-candles and the exposure time was extended beyond 1 hour, the treatment was almost completely lethal, but of the pollen grains that survived by far the largest proportion bore the waxy gene. With one exception (the 1½-hour exposure at 1,200 foot-candles), the temperatures of the treatments were not so high as those of 1931, but the difference in effect between the 2 years is striking.

A direct comparison of the effect of artificial illumination and of treatment in the dark at the same temperature is given in table 14. The ears in each comparison are from identical lots of pollen collected from individual male parents. The pollen samples stood side by side during treatment, the only difference between them being that the dark series were in covered pill boxes. The number of seeds in the light series is unfortunately small, and indeed only two of the ears in this series have any significance by themselves. In the aggregate, however, they emphasize the greater effect obtained with relatively high temperatures in conjunction with light as compared with the effect of similar temperatures in the dark.

TABLE 14.—*Percentage of waxy seeds obtained from pollen subjected to artificial illumination of 1,200 foot-candles and from duplicate samples of pollen stored in the dark*

Treatment		Pollen sample	Seeds from pollen exposed to—			
			Dark		Light	
Duration	Temperature [1]		Total	Waxy	Total	Waxy
Hours	*° C.*	*No.*	*Number*	*Percent*	*Number*	*Percent*
1½	47	2339	2,306	50.0±0.7	3	100
		2353	1,008	68.1±1.0	7	100
		2437	1,272	37.8± .9	122	66.4± 2.9
		2461	1,936	47.0± .8	4	100
		2486	161	84.5±1.9	7	100
		3007	58	84.5±3.2	6	83.3±10.2
2	41	2816	450	81.3±1.2	16	100
		2694	672	92.1± .7	116	100
		2658	2,020	48.0± .8	8	100
		2843	1,862	54.5± .8	10	90 ± 6.4
2	47	3218	422	49.1±1.2	6	100
		3629	43	53.5±5.1	5	100

[1] The temperatures given are the maximum attained during treatment.

INDIVIDUAL PLANT RESPONSES TO POLLEN TREATMENTS

The individuality of the reactions of the various pollen samples to the dark treatment has already been pointed out; a more detailed discussion follows:

As an illustration of the variability of reaction of different samples of pollen, the data presented in table 15 have been assembled. The pollen was collected from four sister plants at 8 a. m., July 30, 1932, and subjected to various treatments. These four plants were chosen for this presentation because the treatments were alike and reasonably successful in all categories. It will be observed that (1) only the pollen produced by plant 3005 showed a reaction to sunlight, (2) only the pollen from plant 2825 failed to react to 47° C. in the dark for 1½ hours, (3) the pollen of plants 2486 and 3005 failed to show any effect of storage for 24 hours, and (4) the pollen of plant 3007 failed to respond to artificial illumination of 1 hour's duration.

The pollen from all plants responded to some treatments, and it is not possible to conclude that the pollen of one plant is more or less sensitive than that of another. Apparently the condition of the pollen determines its response to a particular treatment. Thus pollen from plant 3005 reacted to sunlight, artificial light, and high temperature in the dark, but storage for 24 hours failed to bring about a change. On the other hand, pollen from plant 2825 showed only a relatively slight effect when subjected to artificial illumination but a pronounced reaction to prolonged storage.

Because of their individuality, these responses give little insight into the nature of the reactions causing the differential survival of the two sorts of pollen, but they help to explain the variability encountered in arrays of ears obtained from the use of treated pollen.

TABLE 15.—*Percentage of waxy seeds from pollen collected from 4 plants at the same time, July 30, 1932, and subjected to light and darkness for various periods and at different temperatures*

Treatment				Seeds from pollen collected from plant no. —							
				2825		2486		3005		3007	
Exposure	Duration	Light intensity	Temperature [1]	Total	Waxy	Total	Waxy	Total	Waxy	Total	Waxy
	Minutes	*Footcandles*	*° C.*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Fresh				1,418	45.3±0.9	2,318	44.6±0.7	2,296	45.6±0.7	2,169	48.9± 0.7
Sun	15	4,000	42	1,303	46.9± .9	1,378	48.3± .9	1,245	42.5± .9	1,285	45.6± .9
Do	30	4,400	42	936	43.6±1.1	892	51.0±1.1	945	48.8±1.1	1,240	44.3± 1.0
Do	45	4,400	42	1,278	47.2± .9	555	48.3±1.4	250	61.6±2.1	885	48.0± 1.1
	Hours										
Light	1	800	37	1,347	53.3± .9	18	88.9±5.2	765	59.3±1.2	1,006	51.7± .8
Do	1½	1,200	47			7	100.0			6	83.3±10.3
Dark	1		37					1,353	47.2± .9	1,005	45.9± 1.1
Do	1½		47	1,179	43.1±1.0	161	84.5±1.9	199	73.9±2.1	58	84.5± 3.2
Do	24		26	253	74.3±1.9	277	44.8±2.0	708	45.9±1.3	1,155	64.0± 1.0

[1] Maximum attained during treatment.

DURATION OF STORAGE

SEVENTEEN HOURS

By collecting pollen in the afternoon, using one lot immediately as a fresh control, and applying the remainder the following morning, a storage period of 17 hours was obtained, during which the pollen was kept in covered containers at room temperature.

In this experiment pollen was collected from individual plants at Arlington, Va., at 4 p. m., August 6, 1929. The stored pollen was applied at 9:30 a. m. the following morning, giving approximately 17 hours of storage. In 19 samples the pollinations were successful in providing ears from both the fresh and the stored pollen. The ears obtained from the fresh pollen had the highest percentages of waxy seeds, but the difference of 2.62 ± 1.15 is not significant.

Although there were only 19 successful paired pollinations, 25 ears were obtained from fresh pollen and 26 ears resulted from the use of stored pollen. When these two groups were examined from the standpoint of their female parents there was seen to be strong evidence of the influence of the female parent on the percentage of waxy seeds. The waxy plants used as female parents in this experiment were in two progenies. The plants in one group were designated by numbers between 1200 and 1300; in the other group, by numbers between 500 and 700.

Fresh pollen applied to the plants numbered between 1,200 and 1,300 produced 49.1 percent waxy seeds. Similar pollen applied to plants of the other progeny produced a mean percentage of 47.7 waxy seeds. The two groups are in fair agreement, χ^2 equaling 2.06 ($P = 0.147$) and the mean of the two being 48.4. However, when stored pollen was applied to plants numbered between 1,200 and 1,300, the resulting seed was but 41.96 percent waxy, whereas similarly treated pollen used on plants numbered between 500 and 700 gave ears with a mean of 51.51 percent waxy seeds. The plants furnishing the pollen were not sibs of either progeny used as female parents. The χ^2 of the difference between these two progenies is 7.05 ($P = 0.008$).

In one case storage decreased the effective proportion of waxy pollen grains, whereas in the other case the proportion of these pollen grains was increased. Clearly there is an effect of the female parent on the behavior of stored pollen. No such effect can be demonstrated for the fresh pollen; in fact, the progeny that gave the highest percentage of waxy seeds from the use of stored pollen gave the lowest percentage when fresh pollen was used. The difference between the two progenies in their reaction to stored pollen as compared with fresh pollen is 10.99 ± 2.21. The data are given in table 16.

TABLE 16.—*Effect of female parent on percentage of waxy seeds from fresh and from stored pollen*

Female parent progeny no.	Waxy seeds from indicated pollen		Difference (fresh—stored)
	Fresh	Stored	
	Percent	*Percent*	*Percent*
1200	49.14±0.79	41.96±1.02	7.18±1.29
500	47.70±1.34	51.51±1.20	−3.81±1.80
Difference	1.44±1.55	−9.55±1.57	10.99±2.21

At Sacaton, Ariz., 1929, a short series of plants was self-pollinated, one half with pollen collected and applied in the afternoon, the other half with pollen stored overnight and applied the following morning. The period of storage was 16 hours and 5 minutes and, judging from the few ears and small number of seeds, the lethal limit was closely approximated. The ears obtained from the use of fresh pollen had 18.9 ± 0.6 percent waxy seeds, those obtained from stored pollen had 19.3 ± 0.8 percent waxy seeds. The difference is not significant. Both lots of ears have much lower percentages of waxy seeds than is customary in strains where no disturbing factor such as the *su* gene is present.

Twenty-Four Hours

In 1929, 1931, and 1932 there were a number of instances where pollen was stored in the dark (in covered pill boxes) for 24 hours. In these cases the pollen was collected in the morning and applied the following morning. In 1932 many of these long-period exposures were successful, whereas in other years the majority were failures. The data are presented in table 17. The results are very erratic, with the possible exception of the pollinations made in 1931, where there was a consistent and significant decline in the effective proportion of waxy to horny pollen grains in the stored pollen as compared with the fresh pollen.

Table 17.—*Percentage of waxy seeds obtained from fresh pollen and from pollen stored for 24 hours in the dark at air temperature*

Place	Date	Pollen sample no.	Seeds from indicated pollen				Difference in waxy seed from fresh and from stored pollen
			Fresh		Stored		
			Total	Waxy	Total	Waxy	
			Number	*Percent*	*Number*	*Percent*	
Arlington	Aug. 1–2, 1929	409	267	47.2±2.1	55	100.0	−52.8±2.1
		434	370	56.2±1.7	18	38.9±7.8	17.3±7.9
		701	553	56.2±1.4	45	95.6±2.1	−39.4±2.5
		Av.					−44.9±6.7
Lanham	Aug. 1–2, 1931	609	376	44.4±1.7	221	38.0±2.2	6.4±2.8
		680	421	46.8±1.6	220	36.4±2.2	10.4±2.7
		1857	482	43.6±1.5	167	32.9±2.5	10.7±2.9
		1967	107	61.7±3.2	278	44.2±2.0	17.5±3.8
		1969	543	47.0±1.4	331	43.2±1.8	3.8±2.3
		Av.					8.5±1.4
Do	July 30–31, 1932	2339	1,203	43.7±1.0	534	44.0±1.5	−.3±1.7
		2340	1,378	45.5± .9	410	43.2±1.7	2.3±1.9
		2353	1,055	42.5±1.0	584	46.9±1.4	−4.4±1.7
		2448	1,115	46.1±1.0	289	43.3±2.0	2.8±2.2
		2460	1,006	41.4±1.1	252	42.5±2.1	−1.1±2.4
		2461	1,184	43.0±1.0	385	41.8±1.7	1.2±2.0
		2486	2,318	44.6± .7	277	44.8±2.0	−.2±2.1
		2513	851	47.4±1.2	323	78.6±1.5	−31.2±1.9
		2542	1,168	46.1±1.0	455	39.8±1.6	6.3±1.8
		2638	495	40.4±1.5	682	42.1±1.3	−1.7±2.0
		2825	1,418	45.3± .9	253	74.3±1.9	−29.0±2.1
		3005	2,296	45.6± .7	708	45.9±1.3	−.3±1.4
		3007	2,169	48.9± .7	1,155	64.0±1.0	−15.1±1.4
		Av.					−6.1±2.2

In 1929 and 1932 there were 5 large and unquestionable increases in the percentage of waxy seeds following 24 hours of storage, 3 of

these occurring in 1932. No explanation can be offered as to why these 3 samples behaved so differently from the remaining 10, other than the possible effect of slight differences in quantity of pollen. All of these samples had duplicates that were stored for 1½ hours in the dark at a temperature that reached a maximum of 47° C. Two of these heat-treated duplicates (samples 2448 and 2513) failed to function when used to pollinate waxy plants.

The fact that the heat treatment proved lethal might be urged as indicating a greater sensitivity for these two samples than for the remainder, but 24 hours of storage produced only a negligible change in the proportion of the two sorts of pollen grains in sample 2448, whereas sample 2513 showed a very great change.

Aside from the samples that failed to produce ears, sample 2460 had a period of storage in the sun for 1 hour and a duplicate sample had a covered exposure in the sun designed to equalize temperature effects. The exposure to direct sunlight proved lethal, but the covered sample produced 10 ears showing no alteration in the proportion of the two sorts of pollen grains, the temperature in this case having attained a maximum of 50° C. All the other samples, with the exception of samples 2340, 2353, and 2825, gave significant increases when stored for 1½ hours in the dark at 47°. Apparently the exceptions, 2340, 2353, and 2825, were not sensitive to the treatment, yet sample 2825, when stored for 24 hours, showed a large increase in the functioning proportion of waxy to horny pollen grains.

These 24-hour periods of storage are of interest chiefly in showing that in some instances the processes that effect the differential functioning of these two sorts of pollen grains take place with the passage of time in the absence of light and at ordinary temperatures.

EFFECT OF POLLEN STORAGE ON PERCENTAGE OF WAXY SEEDS IN BUTTS AND TIPS

If certain storage conditions gradually reduce the vitality of the pollen grains so that the tubes of weakened grains grow more slowly than those of fully vital grains and if this reduction in vitality takes place faster in horny than in waxy grains, then the proportion of waxy to horny seeds in the butts of ears pollinated with stored pollen should be higher than that found in the tips of the same ears.

In bulk populations where classifications were made separately for the butts and tips, it was found that ears resulting from fresh pollen had 43.7 ± 0.6 percent waxy seeds and that ears resulting from pollen stored in the light for 7½ hours had 48.4 ± 0.8 percent waxy seeds, a difference of 4.7 ± 1.0 due to storage. The increase is not great but it is significant and of sufficient magnitude to show an effect of treatment. Unfortunately, when these pollen samples were treated there was no direct sunlight, the day being cloudy and overcast, with high humidity. This may be the explanation of the slight increase in effectiveness of waxy pollen grains as well as of the unusual number of successful pollinations obtained with stored pollen. Although the observed alteration in the effective proportion of the two sorts of pollen grains is low, certain pertinent information may be derived from a comparison of the percentage of waxy seeds in the butts with that in the tips of the ears.

In a group of 56 ears resulting from the use of stored pollen, the difference between the percentage of waxy seeds in the butts and that

in the tips was 3.1 ± 0.4 percent. In a group of 31 ears resulting from the use of fresh pollen, the difference between the percentage of waxy seeds in the butts and that in the tips was 0.7 ± 0.5. In both cases the butts had the higher percentage of waxy seeds. The difference between these differences is 2.4 ± 0.7, which may be considered significant. No such difference between butts and tips in the percentage of waxy seeds is found when pollen from homozygous waxy plants is used on ears heterozygous for the waxy gene. In this same population the plants that furnished the heterozygous pollen for storage treatments were pollinated with homozygous waxy pollen, and the seeds from the butts and from the tips were separately classified. There were 118 of these ears, with a mean difference between butts and tips of 0.3 ± 0.3 in the percentage of waxy seeds, the butts having the higher percentage.

In the comparison between the percentage of waxy seeds in the butts and in the tips of stored and fresh pollen, not all of the ears examined came from identical pollen samples, for in some cases the fresh half of the pollen sample produced ears when the stored half failed, and in other cases the reverse was true. There were, however, 17 cases where paired pollinations produced ears, one from the fresh and the other from the stored half of the pollen. Restricting the comparison of the percentage of waxy seeds in the butts and in the tips to these 17 pairs of ears, it is found that the percentage of waxy seeds in the butts is higher than that in the tips in both lots of ears and that the difference between the differences is 1.6 ± 0.9 percent, the greater difference being found where the ears resulted from the use of stored pollen. This is not a significant difference.

If the differences observed in the bulk populations are due largely to reduction in pollen vitality by conditions operating during storage, it should be found in this population that the ears with fewest seeds had the greatest differences between butts and tips in the percentage of waxy seeds. This relationship would not be very close, as many factors other than pollen vitality influence the total number of seeds produced on an ear. The product-moment coefficient of correlation between the number of seeds and the magnitude of the difference in percentage of waxy seeds between butts and tips is found to be -0.245, indicating that, as the number of seeds increased, the percentage of waxy seeds in the butts and tips tended to reach equality. This coefficient, however, is not significant, P falling between 0.1 and 0.05.

In the bulk populations, it is found that in the group of 56 ears resulting from stored pollen there is a correlation of 0.701 ± 0.046 between the percentage of waxy seeds in the butts and that in the tips. In the group of 31 ears resulting from the use of fresh pollen this correlation is 0.628 ± 0.075. In the group of 118 ears resulting from the use of homozygous waxy pollen on ears heterozygous for waxy, the correlation is -0.017 ± 0.062. Clearly, the relationship between the two halves of the ear in percentage of waxy is much closer where pollen is involved than where the distribution of seeds is determined by the formation of the ovules.

Since in this material each main sample represented a single heterozygous plant, it might be argued that the plants differed with respect to the production of viable waxy pollen grains. It is more probable, however, that the viability of pollen was changed by some condition subsequent to anthesis. With stored pollen, where an

artificial difference has been interposed between the several lots of pollen the intraclass correlation is marked. It should be borne in mind that in the two series of ears resulting from heterozygous pollen essentially the same plants are involved as pollen parents. The stored pollen was identical with that of fresh pollen insofar as it was possible to insure identity. However, it is equally clear that although the pollen samples were stored in the sunlight under as nearly identical conditions as possible some condition was present that served to differentiate the separate lots.

Clearly something operates during storage that tends to make the ears pollinated by a subsample from a given lot of pollen more nearly alike than would be expected from chance alone. In other words the lots of stored pollen have become differentiated. This could be explained on the assumption that the samples differed in moisture content at the time of collection, a condition that would have no effect on the pollen used immediately but might be an important factor in its reaction to storage. Many other explanations suggest themselves, e. g., that some lots contained more pollen than others, thus providing a variable pollen screen affording the lower layers a variable protection from the sun. Variation in quantity would have little effect on pollen applied immediately. Speculation as to the cause or causes underlying the observed differences in degree of intraclass correlation are futile, but the existence of these differences is further evidence that storage (whatever the factors involved) has a differential effect on these two sorts of pollen grains.

DISCUSSION

Although the storage experiments conducted with pollen samples from maize plants heterozygous for the waxy gene have not resulted in demonstrating any regularity of behavior of pollen grains, there is no question that the proportions of the two sorts of grains functioning in fertilization can be altered by external agencies. It is not possible to outline at this time a treatment of pollen that will produce a given percentage of waxy seeds, but it may be stated with certainty that exposure to light will usually result in an increased effectiveness of waxy pollen grains. Were it not for the results obtained with composite pollen samples in one season, a like conclusion could be reached with respect to heat treatments in the absence of light. As the data now stand, heat treatments may either increase or decrease the effectiveness of waxy pollen. Under the circumstances, it seems more logical to assume that some factor other than the treatments given caused the deficiency in the composite samples, in which case it might be assumed that heat in the absence of light also increases the effectiveness of waxy pollen grains.

Whatever process takes place in the treated pollen grains it has been demonstrated that in some instances time can be substituted for light or heat, with similar results. The fact that storage in the dark at ordinary temperatures sometimes brings about an unequal functioning of the two sorts of grains points in the direction of an oxidation process that is speeded by illumination or high temperatures.

Brink (*3*) has shown that the waxy carbohydrate has a lower diastatic activity than ordinary cornstarch, and in harmony with this finding Sprague (*21*) shows that pollen grains containing waxy starch

require more time for germination than do grains containing ordinary starch. If fresh waxy and horny pollen grains are applied to stigmas in quantities far in excess of the number of ovules, the result of the differential rate of germination will be a deficiency of waxy seeds. However, if small quantities of pollen were used the difference between waxy and horny pollen in the time required for germination would not be reflected in the seed classes, since competition for ovules would be largely eliminated.

If storage conditions operate merely to kill most of the pollen grains, then pollinations made with stored samples would in effect be made with greatly reduced numbers of effective grains and the two types of seed should approximate equality more nearly than when fresh pollen is used.

To account for a deficiency of waxy seeds from the use of fresh pollen and an excess from the use of stored pollen, it has been suggested (*12*) that the two sorts of grains were not equally mature at anthesis (a condition that might also explain the difference in time required for their germination) and that before waxy grains have reached maturity death has begun to eliminate horny grains.

Sprague's experiments offer the possibility that the conditions of storage overcome whatever factors operate to delay germination of the waxy grains and that the rate of germination of grains of this type is actually accelerated beyond that of the horny grains. This explanation may be merely substituting germination for maturity, but its greater specificity makes it attractive.

In this connection the experiments of Flint (*9*) on the role of light in the germination of lettuce seeds are of interest. Flint shows that light in the red end of the spectrum is essential for germination in certain samples of seed and that any sample of lettuce seed can be made to require red light for germination by a short exposure to radiation in the blue end of the spectrum.

Although it is a far cry from the germination of lettuce seeds to the differential functioning of two sorts of pollen grains of maize, it is quite possible that similar photochemical processes are involved. Flint's work shows that the wave lengths affecting germination in lettuce are close to if not identical with those that affect the development of rancidity in oils and that these regions of the spectrum have an important function in photosynthesis.

It may be that the photochemical process which differentiates between waxy and horny pollen grains is operating to oxidize fats, in which case the waxy gene must have an effect on the type or quantity of stored fats in addition to its well-recognized effect on the type of starch.

The chief drawback to this hypothesis is found in the loss of vitality of stored pollen grains. That storage conditions do kill most of the pollen grains hardly needs affirmation, but that the death rate differentiates between the two types of pollen can be inferred only from the results.

Attempts to estimate the correlation of the loss in germinating power with the time of exposure to deleterious conditions by germinating pollen samples on excised silks have not been very successful, although the tests show clearly that vitality declines. With uninjured pollen a casual inspection of silks a few minutes after pollination is enough to disclose many germinated grains, but with

treated pollen much hunting about may be required before a single germinated grain is found. Of course there is no method of distinguishing the waxy from the horny grains in a heterozygous sample without destroying their vitality. It is, therefore, not possible to measure directly any differential change in rate of germination between these two kinds of pollen grains that might result from storage.

Information on this point is very limited, for germination tests of pollen were made simply to obtain an immediate check on the lethal effect of treatments. Pollinated silks were examined and records made as to whether or not germinated grains were found. If no such grains were discovered 15 minutes after pollination, another sample was examined in half an hour, and this scrutiny was resumed at half-hour intervals up to 3 hours. No effort was made to arrive at percentages of germination, the records simply noting relative degrees of germination. These records do show, however, that some samples in which no germinated grains could be found after the first 15 minutes did in fact have a few germinated grains after 2 hours. Such observations lend little support to the hypothesis that storage treatments accelerate germination.

Although, in general, stored pollen showed less germinated grains than fresh pollen and a slower rate of germination, as measured by the elapsed time before germinated grains could be found, the method is not wholly satisfactory. In several cases good ears were produced with pollen showing no germination on excised silks, and in other cases the reverse was true.

Attempts to find a physical difference between waxy and horny pollen grains were without success. This is rather surprising, as it seems improbable that such a distinctive chemical difference would not be reflected in some measure of size. Indeed, in the seeds a difference is found in weight (waxy seeds being lighter), and since there is no difference in specific gravity waxy seeds are smaller in volume than horny seeds from the same ear (*7*). The difference in volume between these two sorts of seeds approximates 5 percent of the waxy seeds. Had a similar difference existed in the pollen grains it should have been disclosed in the tests with sieves and the micrometer measurements. The errors in the determinations of size of pollen grains were such that differences of the order of 3 percent could have been detected.

Although it appears impossible to separate waxy from horny pollen grains by physical means, Mangelsdorf (*16*, *17*) has shown such separations to be possible with other differentiating genes in maize, and the process may be a general one in constant operation. With the brief time required for the germination of maize pollen (*21*), even slight differences in size or specific gravity (which would affect the time of their deposition on the stigmas) might have selective importance.

In the case of the waxy gene enough evidence has been presented to show that under certain adverse conditions this gene provides a survival factor for the pollen grains carrying it. There can be little doubt that under the proper conditions only waxy pollen grains would survive to effect fertilization, and probably there are many other genes that condition similar reactions. With the enormous populations of pollen grains provided by every corn plant, an oppor-

tunity is present for rigorous selection, although there is no evidence that the pollen grains surviving unfavorable environments contribute unusual vigor to the resulting zygotes.

SUMMARY AND CONCLUSIONS

No differences in size, weight, or specific gravity were found between waxy and horny pollen grains.

There is some evidence of a deficiency of functioning waxy pollen grains among the grains of smallest size.

There is no evidence that waxy pollen grains are less mature when shed early in the morning than when released later in the day.

Exposure of pollen grains from plants heterozygous for the waxy gene to direct sunlight for several hours increases the proportion of waxy grains that effect fertilization in those samples where this treatment is not lethal.

Similar though more erratic results are obtained when such pollen is stored in the dark for 1 or 2 hours at temperatures exceeding 40° C.

Exposure for 1 hour to artificial light of 0.1 the intensity of direct sunlight increases the proportion of waxy pollen grains that function in fertilization.

There is some indication that storage at high temperature and also in oxygen reduces the proportion of waxy pollen grains that can effect fertilization, but since this result has been obtained only with a single composite pollen sample collected from many plants it is thought that the method of compounding the pollen, rather than the storage treatment, was the causal factor.

The greatest change in the relative effectiveness of waxy pollen grains is found when pollen is exposed both to high temperatures and to light.

Storage in the dark at low temperatures (10° C.) produces no measurable effect.

Pollen samples collected from individual plants were found to react differently to the several treatments.

Pollen collected in the afternoon and applied the following morning showed no change in the relative effectiveness of waxy and horny grains.

Pollen collected in the morning and held in the dark for 24 hours at room temperature occasionally showed an increased effectiveness of the waxy grains.

When stored pollen is used the percentage of waxy seeds is higher in the butts than in the tips of the ears, indicating a relative reduction in the vitality of horny pollen grains.

A single population was obtained that showed conclusively an effect of the female parent on the behavior of pollen grains subjected to storage. The source of pollen was the same for all ears, and the ears resulting from the use of fresh pollen had similar percentages of waxy seeds, all slightly in defect of equality. In one of the groups of ears resulting from the use of stored pollen, the percentage of waxy seeds was less than that obtained with fresh pollen, whereas in the other group of ears stored pollen resulted in a higher percentage of waxy seeds than was obtained with fresh pollen.

LITERATURE CITED

(1) Bond, C. J.
1927. the influence of pollen maturity and restricted pollination on a simple mendelian ratio in the pea. Jour. Genetics 17: [269]–281, illus.

(2) Brink, R. A.
1925. mendelian ratios and the gametophyte generation in angiosperms. Genetics 10: 359–394, illus.

(3) ———
1929. studies on the physiology of a gene. Quart. Rev. Biol. 4: 520–543, illus.

(4) Chao, L. F.
1928. the disturbing effect of the glutinous gene in rice on a mendelian ratio. Genetics 13: 191–225, illus.

(5) Christie, W., and Wriedt, C.
1930. seasonal effects on mendelian segregations and sex ratios. (Written by H. Wexelsen.) Hereditas 14: [173–196.]

(6) Ciesielski, T.
1912. how it happens that the offspring of plants, animals, and men is sometimes male, sometimes female. Internatl. Clinics (22) 3: 5–16.

(7) Collins, G. N., and Kempton, J. H.
1913. inheritance of waxy endosperm in hybrids with sweet corn. U. S. Dept. Agr. Circ. 120: 21–27, illus.

(8) ——— and Kempton, J. H.
1927. variability in the linkage of two seed characters of maize. U. S. Dept. Agr. Bull. 1468, 64 pp., illus.

(8*a*) Correns, C.
1924. über den einfluss des alters der keimzellen i. Sitzber. Preuss. Akad. Wiss. 9: 70–103.

(9) Flint, L. H.
1934. light in relation to dormancy and germination in lettuce seed. Science (n. s.) 80: 38–40.

(10) Gotoh, K.
1931. physiological researches on pollen with special reference to the artificial germination of gramineae pollen. Mem. Facult. Sci. and Agr., Taihoku Imp. Univ. 3: [61]–197, illus.

(11) Haigh, J. C., and Lochrie, J. V.
1929. investigation of a mendelian ratio in vigna sinensis by a consideration of the progeny from successive daily crosses. Ann. Bot. [London] 43: [783]–803, illus.

(11*a*) Holman, R. M., and Brubaker, F.
1926. on the longevity of pollen. Calif. Univ. Pubs., Bot. 13: 179–204.

(12) Kempton, J. H.
1927. age of pollen and other factors affecting mendelian ratios in maize. Jour. Agr. Research 35: 39–74, illus.

(13) Knowlton, H. E.
1922. studies in pollen, with special reference to longevity. N. Y. (Cornell) Agr. Expt. Sta. Mem. 52, pp. 751–793.

(14) Malhotra. R. C.
1930. can sex ratio be altered in dioecious plants? Amer. Nat. 64: 470–473.

(15) ———
1930. the sex ratio in asparagus officinalis l. and its artificial modification. Jour. Genetics 23: [157]–172, illus.

(16) Mangelsdorf, P. C.
1931. modification of mendelian ratios in maize by mechanical separation of gametes. Natl. Acad. Sci. Proc. 17: 698–700.

(17) ———
1932. mechanical separation of gametes in maize. Jour. Heredity 23: 289–295, illus.

(18) Nakamura, M.
1932. daily changes in the occurrence of the imperfect pollen grains in impatiens balsamina linn. Jour. Soc. Trop. Agr. Japan 4: 149–160, illus.

(19) SASAHI, T.
1925. ON THE PRESERVATION OF THE POLLEN OF CEREALS. Jour. Sci. Agr. Soc. 275: 259–287. [English abstract in Japan. Jour. Bot. 3: (22)–(23). 1926.]
(20) SISA, M.
1932. RELATIONSHIP BETWEEN AGE AND THE VIABILITY OF POLLEN IN DIFFERENT CUCURBITS. Japan. Jour. Genetics 8: 19–26.
(21) SPRAGUE, G. F.
1933. POLLEN TUBE ESTABLISHMENT AND THE DEFICIENCY OF WAXY SEEDS IN CERTAIN MAIZE CROSSES. Natl. Acad. Sci. Proc. 19: 838–841, illus.

WI

By F
Di
De

F
year
year
of t
in a
oat,
ized
lem

T
to s
the
Fulg
mor
vari

T
Ave
well
attr
form
Sinc
natu
mor
mut
has
pop
addi
natu
and
betw

T
fatu
fere
com
Ave
norr

[1] Re
[2] Re

Journa
Wash

WIDESPREAD OCCURRENCE AND ORIGIN OF FATUOIDS IN FULGHUM OATS[1]

By F. A. COFFMAN, *associate agronomist*, and J. W. TAYLOR, *associate agronomist*, *Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Fatuoids in oats (*Avena* L.) have been recognized for about 50 years (*13*, *20*)[2] and have been of especial interest for more than 25 years because of (1) their economic importance and (2) the nature of their origin. At present there is more interest in the fatuoid than in any other aberrant in the small grains. The fatuoid, or false wild oat, usually is similar to the variety in which it occurs and is characterized by lemmas having awns and basal characters similar to the lemmas of *A. fatua* L.

The present paper is a further report on a study by the writers (*3*) to show the distribution of the fatuoid in the Fulghum variety and the role played by natural crossing in the occurrence of fatuoids. Fulghum was chosen because it is generally recognized as containing more fatuoids than any other agriculturally important American oat variety.

REVIEW OF LITERATURE

The possibility of fatuoids becoming economic weeds, such as *Avena fatua*, has been mentioned by several investigators (*8*, *28*) as well as in press dispatches. The origin of fatuoids is commonly attributed either to natural crossing (*1*, *29*, *30*, *31*, *32*, *33*) or to a form of mutation (*3*, *5*, *6*, *8*, *9*, *10*, *14*, *15*, *16*, *17*, *18*, *19*, *22*, *23*, *24*, *25*). Since 1907 the mutation theory has gained many adherents and the natural crossing but few. However, in 1929, Tschermak (*31*), after more than 15 years of study of the problem, asks proof from the mutationists as to the purity of their populations. That Tschermak has grounds for questioning the purity of an open-pollinated oat population is known from natural-crossing studies (*11*, *12*, *27*). In addition, Coffman and Wiebe (*4*) have observed examples of apparently natural crossing between *A. sativa* and *A. fatua*, and Aamodt, Johnson, and Manson (*1*) have conducted extensive studies of natural crossing between these two species.

The results of inheritance studies so far reported indicate that fatuoids are recessive to normal oats, usually by a single-factor difference. The writers have observed this to be true in crosses of the common, or "A type", fatuoid in Fulghum with varieties of either *Avena sativa* L. or *A. byzantina* C. Koch. The intermediate and normal forms are inseparable in some crosses.

[1] Received for publication Sept. 16, 1935; issued March 1936.
[2] Reference is made by number (italic) to Literature Cited, p. 130.

PREVALENCE OF FATUOIDS

The cultivated red oat (*Avena byzantina*), to which Fulghum belongs, is considered by Coffman, Parker, and Quisenberry (*2*) to be a derivative of *A. sterilis* L., and at least two varieties belonging to that species, namely, Burt and Fulghum, contain many diverse forms, the origin of which is difficult to explain. The difficulty in obtaining true-breeding Fulghum has been reported by Stadler and Kirkpatrick (*26*), Parker, as reported by Farrell (*7*), and others. Progenies of single Fulghum panicles frequently contain fatuoids 2 or 3 years after selection, and possibly no selection of extensively grown Fulghum is free from fatuoids.

The information on the prevalence of fatuoids presented in table 1 was obtained at 28 experiment stations in 17 States of the United

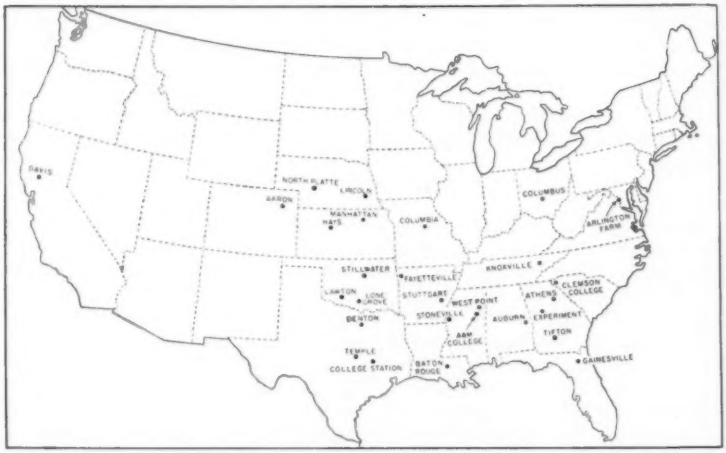

FIGURE 1.—Location of experiment stations in the United States where the presence of fatuoids in Fulghum oats has been investigated.

States (1) by questionnaires sent to experiment stations, (2) by examination of seed samples of Fulghum oats received from these stations, (3) by growing and observing plants grown from this seed at Arlington Experiment Farm, Rosslyn, Va., and (4) by observations made at the experiment stations themselves. As shown in figure 1, stations in all of the important Fulghum-producing States were included in this survey.

In 19 of the 25 samples of seed received from 18 stations, including Arlington Farm, fatuoids were found ranging from 0 to 12 per 2,000 kernels counted and averaging 0.11 percent for the 50,000 kernels examined.

When these seed samples were sown in plots in 1930, it was observed that 20 of the 25 seed samples produced some fatuoid plants. The number ranged from 0 to 9 per 2,000 individuals and averaged 0.22 percent in the 41,022 plants observed. The following year a second seeding was grown from 20 of these 25 samples. Progeny from 8 of these samples contained fatuoids, ranging from 0 to 0.67 percent per

plot. Poor germination resulted in thin stands, but, among 11,924 plants examined, 16, or 0.13 percent, were fatuoids.

TABLE 1.—*Summary of data on occurrence of fatuoids in Fulghum oats at various experiment stations during several seasons*

Item	Stations represented	Samples submitted	Reports or observations on—			Individuals examined	Fatuoids found		
			Fatuoids present	Fatuoids absent	Total				
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent* [1]	*Percent* [2]
Questionnaires	17		13	4	17				
Seed samples:									
Fall-sown	8	12				24,000	22	0.092	
Spring-sown	10	13				26,000	33	.127	
Total	18	25	19	6	25	50,000	55	.110	0-0.60
Plants grown:									
Arlington Experiment Farm, 1930: [3]									
Fall-sown	8	12				18,734	37	.198	
Spring-sown	10	13				22,288	52	.233	
Total	18	25	20	5	25	41,022	89	.217	0- .45
Arlington Experiment Farm, 1931: [4]			8	12	20	11,924	16	.134	0- .67
Experiment stations, 1931: [5]									
Fall-sown	14	14				12,800	42	.328	
Spring-sown	4	4				3,500	22	.629	
Total	18	18	18	0	18	16,300	64	.393	.10- .90
Total fall-sown [6]						55,534	101	.182	
Total spring-sown [7]						51,788	107	.207	
Grand total [8]						119,246	224	.187	

[1] Average.
[2] Range.
[3] 25 samples of seed from 18 experiment stations (including Arlington) were sown at the Arlington Experiment Farm, Rosslyn, Va.
[4] Second seeding of 20 of the seed samples made at Arlington Experiment Farm.
[5] Independent observations made at 18 southern experiment stations.
[6] Includes fall-sown seed and plants.
[7] Includes spring-sown seed and plants.
[8] Includes total fall-sown, total spring-sown, and plants grown at Rosslyn, Va., in 1931.

Observations made on 18 southern experiment stations in the summer of 1931 disclosed the presence of fatuoids at all stations. The number ranged from 1 to 9 per 1,000 individuals and averaged 0.39 percent of the 16,300 plants examined.

As a result of several years' studies it was found that a few fatuoids were present in Fulghum at all 28 stations in 17 States; that they were not more prevalent in fall-sown than in spring-sown oat areas; and that the number never equaled 1 percent at any station and averaged only 0.19 percent in 119,246 individual seeds or plants. It may be concluded, therefore, that fatuoids are not now weeds in the United States, nor are they likely to become weeds under present cultural conditions.

ORIGIN OF FATUOIDS

CONSTANCY OF FULGHUM WHEN SELFED

Selfing studies were conducted to measure the importance of natural crossing on the constancy of Fulghum and fatuoids. Individual pan-

icles were enclosed in glassine bags before flowering and the progenies from such panicles were grown.

Results from selfing studies begun in 1927 on Fulghum are presented in table 2. In 1927, 97 Fulghum plants were selfed. These furnished material for later studies. The number of progenies in which no fatuoids occurred after 1, 2, 3, and 5 years of selfing, respectively, were as follows: In 1928, 300; in 1929, 220; in 1930, 216; in 1932, 88. However, of 930 Fulghum individuals grown in 1931, the progeny of 4 years of selfing, 3 were homozygous fatuoids. In 1,754 individuals produced in 5 years, 3, or 0.2 percent, were fatuoids.

The infrequent occurrence of fatuoids indicated that larger populations were desirable, and therefore Fulghum lines open-pollinated for a single season after previous bagging were grown a second year.

No fatuoids due to natural crossing would be expected in Fulghum lines open-pollinated for but 1 year, as the fatuoid is recessive to Fulghum.

TABLE 2.—*Occurrence of aberrant individuals in self- and open-pollinated Fulghum oats at Arlington Experiment Farm, Rosslyn, Va., in the years 1928–32*

Year examined [1]	Normal (previously selfed) [2]				Fatuoid progeny plants					
	Parent plants	Progeny plants			Previously selfed			Previously open-pollinated		
		Total	Fatuoids		Total	Aberrant		Total	Aberrant	
	Number	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
1928	20	300	0	0.0	340	0	0	1,402	16	1.14
1929	9	220	0	.0	105	0	0	132	2	1.51
1930	10	216	0	.0	422	0	0	162	5	3.09
1931	98	930	3	.3	168	0	0	676	46	6.80
1932	8	88	0	.0	21	0	0	587	275	46.85
Total	145	1,754	3	.2	1,056	0	0	2,959	344	11.6

[1] Aberrances observed are the result of mutations or natural crosses that occurred the previous year.
[2] Less than 0.5 percent of aberrant plants were observed in the open-pollinated Fulghum.

In 1930 there were grown 1,198 progeny individuals, the parents of which had been bagged for two generations, i. e., 1927 and 1928, but had been open-pollinated in 1929. In 1931 there were grown 9,856 progeny individuals, the parents of which had been bagged in 1927, 1928, and 1929 but had been open-pollinated in 1930. Six fatuoids were observed in the 1,198 individuals produced in 1930, and 20 in 1931, a total of 26, or 0.2 percent, in the 11,054 plants. The 1,198 progeny plants produced in 1930 were from head-to-row seedings and 6 of the rows contained fatuoids. In 1931 a progeny row was grown of each individual in these 6 rows and the resulting test proved that the 6 strains were similar in breeding behavior to F_3 populations of a cross of normal $\times$ fatuoid which differed by a single factor. As natural crossing was excluded by selfing in 1928, it is apparent that chromosomal irregularities occurred that year, giving in 1929 the intermediate type that segregated in 1930.

All six mutations observed in 1930 apparently were to the intermediate form, although Marquand (*21*) and Huskins (*17*) have reported the observation of mutation direct to the homozygous form. This latter is possible when aberration occurs in both the male and the female sex cells that form the zygote.

However, if fatuoids arise only by mutation and 0.2 percent of these aberrances occur in a population, it would theoretically require 15,625 individuals for the mutation to occur directly to the homozygous form, whereas mutation to the heterozygous form would occur once in each 125 individuals.

CONSTANCY OF THE FATUOID

It has been reported that the Fulghum fatuoid does not always breed true (*7*, *28*). Plants of fatuoids were selfed to test their behavior under controlled pollination. Progenies were grown from the selfed fatuoid panicles as follows: 340 in 1928, 105 in 1929, 422 in 1930, 168 in 1931, and 21 in 1932. The progeny of all these 1,056 bagged fatuoids bred true.

In addition to the selfing studies, progenies from fatuoids of the same lines were grown open-pollinated. Adjacent to the fatuoid lines were rows of Fulghum and Black Mesdag. The F_1 of a natural cross of these varieties on the fatuoid is easily recognized by the dominance of their characters. Intermediate or F_1 plants were observed as follows: In 1928, 16 of 1,402; in 1929, 2 of 132; in 1930, 5 of 162; in 1931, 46 of 676; and in 1932, 275 of 587. Of 2,959 individuals, 344, or an average of 11.6 percent, apparently were crosses. In 1932, more than 46.8 percent of the progeny of open-pollinated fatuoids apparently were crosses. Therefore, it may be stated that the common or A type fatuoid in Fulghum shows a varying tendency to be cross-pollinated unless foreign pollen is excluded.

The high frequency of cross-pollination in Fulghum fatuoids is striking when compared with the low frequency in normal Fulghum. Less than 1 percent of cross-pollination was observed in the normal Fulghum in these experiments as compared with an average of 11.6 percent and a maximum of nearly 47 percent in the fatuoids in a single season. Of course, factors of climate and soil may influence pollination.

VIGOR OF THE FATUOID

Several investigators have stated that there was little difference in vigor between the normal and the A type Fulghum fatuoid. The data in tables 3 and 4 indicate that plants of fatuoid Fulghum are shorter than those of normal Fulghum and bear more than twice as many infertile florets. The average height of the fatuoids was 38.8 ± 0.06 inches; of the intermediate fatuoids, 40.6 ± 0.06 inches; and of the apparently normal plants, 40.7 ± 0.09 inches. It is not always possible to differentiate the intermediate fatuoid from the normal plant.

TABLE 3.—*Relative height of normal, intermediate, and fatuoid Fulghum plants in segregating populations*

Type of plant	Plants	Height of plant	Tillers per plant
	Number	*Inches*	*Number*
Normal	136	40.7±0.085	3.5±0.069
Intermediate [1]	249	40.6± .062	3.9± .061
Fatuoid	107	38.8± .064	4.0± .124

[1] It is often difficult to separate intermediate and normal forms.

TABLE 4.—*Relative fertility of florets on open-pollinated, primary, secondary, and self-pollinated panicles of normal and fatuoid Fulghum oats, 1927–31*

Crop year	Florets on normal panicles of indicated type						Florets on fatuoid panicles of indicated type					
	Primary		Secondary		Selfed [1]		Primary		Secondary		Selfed [1]	
	Total	Sterile	Total	Sterile	Total	Sterile	Total	Sterile	Total	Sterile	Total	Sterile
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1927	2,329	5.8	1,236	16.7	751	30.2	1,345	11.5	1,330	35.7	2,236	43.9
1928	482	19.7	510	28.4	338	29.6	162	51.2	493	75.3	879	49.1
1929	503	7.8	143	18.9	371	33.2	302	34.1	167	58.1	621	74.4
1930	340	9.4	220	30.5	805	34.8	103	41.7	117	29.1	978	77.2
1931	241	7.0	145	49.7	231	44.6	149	25.5	407	52.1	353	83.6
Total or weighted average	3,895	8.2	2,254	23.0	2,496	33.4	2,061	20.5	2,514	47.3	5,067	57.7

[1] Panicles of primary type.

In the fatuoid and the apparently normal Fulghum plants, 20.5 and 8.2 percent, respectively, of the florets of primary panicles were sterile. In both types of plants more than twice as many florets were sterile in secondary panicles as were sterile in primary panicles. Also, more than twice as many florets were sterile in the primary and secondary panicles of the fatuoid plants as were sterile in these respective panicle types in the normal Fulghum. This high percentage of sterility in fatuoids probably is one reason why Fulghum fatuoids cross-fertilize so frequently and often do not breed true when open-pollinated.

Data obtained on Black Mesdag, grown to compare its fertility with that of Fulghum and the fatuoids, indicated that bagging did not seriously impair fertilization in this *Avena sativa* variety. Data were obtained in each of 4 years. Of a total of 1,563 florets on primary and 647 on secondary panicles, an average of 21.6 and 41.7 percent of the florets were sterile on the primary and secondary panicles, respectively. The range in sterility was from 2.7 percent in 1931 to 34.3 percent in 1930 in the primary and from 13.6 in 1929 to 77.0 percent in 1930 in the secondary panicles. Panicles were bagged in 1928 and 1930. An average of 19 percent of 667 florets were sterile, i. e., 14.3 percent in 1928 and 20.8 percent in 1930.

The fact that fatuoids appear to be slightly less vigorous than normal Fulghum plants probably explains the observed decrease in the number of fatuoids in fall-sown Fulghum and Kanota varietal plots grown at Arlington Experiment Farm. At probably no experiment station included in this study are Fulghum and Kanota grown from fall seeding under conditions so apt to result in winter-killing as at Arlington Farm. The decrease in the number of fatuoids to the one-fortieth-acre plot is indicated by the data in table 5. No data are available on the number of plants per plot, but the trend seems to be very pronounced.

TABLE 5.—*Occurrence of fatuoids in Kanota and Fulghum oats grown at Arlington Experiment Farm, Rosslyn, Va., from seed received from Kansas*

Variety	Date introduced	Fatuoids per 1/40-acre plot in—				
		1923	1926	1927	1928	1929
		Number	*Number*	*Number*	*Number*	*Number*
Kanota	1922	20.0	0.8	1.0	0.3	1.7
Fulghum	1925	----------	14.0	15.0	2.3	1.7

SUMMARY AND CONCLUSIONS

Interest in fatuoids apparently has existed for 50 years and controversy as to their origin for more than 25 years. Of the two explanations offered for their origin, that of mutation is more widely accepted than that of natural crossing between *Avena fatua* and cultivated oats.

Fatuoids were present in Fulghum oats at 28 experiment stations in 17 States of the United States and were not more numerous in the fall-sown than in the spring-sown oat areas. They were nowhere sufficiently numerous to be an economic factor as weeds. They constituted less than 1 percent of any population of Fulghum oats examined. Fatuoids are detrimental chiefly because their presence may discredit otherwise promising oat strains or seed samples.

Approximately 0.2 percent of fatuoids appeared spontaneously in lines of normal Fulghum that had been self-pollinated for four generations. In all cases the mutation apparently occurred to the intermediate type, resulting, in the following generation, in the production of fatuoid and normal plants in a ratio of 1 : 3. When selfed, the Fulghum fatuoid bred true.

Theoretically, the mutation occurred to the intermediate form once in 125 individuals. Mutation to the homozygous form would be expected to occur once in 15,625 individuals.

Data obtained from 5 years' study show that when the fatuoid was open-pollinated it was cross-fertilized on an average 11.6 percent and that a maximum of nearly 47 percent of cross-fertilization occurred in a single season. Under similar conditions, normal Fulghum, recognized as being relatively subject to natural crossing, contained less than 0.5 percent of crosses in any season.

The data indicate that the ordinary or A type fatuoid in Fulghum is less vigorous and more readily cross-fertilized than is the normal plant. This may partly account for the susceptibility of the fatuoid to cross-pollination and may be considered evidence that the persistence of fatuoids in normal oats is due in part to natural crossing.

Covering the panicles with glassine paper bags to prevent cross-pollination reduced fertility in all cases. The percentage of decrease was least in Black Mesdag and greatest in the fatuoid.

Fatuoids of the common or A type are slightly less vigorous than are normal Fulghum plants. This may explain the apparent reduction in number of fatuoids per plot of Fulghum grown on Arlington Experiment Farm from fall seeding.

50929—36——4

LITERATURE CITED

(1) Aamodt, O. S., Johnson, L. P. V., and Manson, J. M.
1934. natural and artificial hybridization of avena sativa with a. fatua and its relation to the origin of fatuoids. Canad. Jour. Research 11: 701–727, illus.

(2) Coffman, F. A., Parker, J. H., and Quisenberry, K. S.
1925. a study of variability in the burt oat. Jour. Agr. Research 30: 1–64, illus.

(3) ——— and Taylor, J. W.
[1932.] prevalence and origin of fatuoids in fulghum oats. (Abstract) Sixth Internatl. Cong. Genetics (Ithaca) 2: 28–29.

(4) ——— and Wiebe, G. A.
1930. unusual crossing in oats at aberdeen, idaho. Jour. Amer. Soc. Agron. 22: 245–250, illus.

(5) Criddle, N.
1909. the so-called white wild oats and what they are. Ottawa Nat. 23: 127–128.

(6) ———
1912. wild oats and false wild oats. their nature and distinctive characters. Canada Dept. Agr. Seed Branch Bull. S–7, 11 pp., illus.

(7) Farrell, F. D.
1924. a genetic study of aberrant and false wild types in kanota oats. Kans. Agr. Expt. Sta. Rept. 1922–24: 38–41.

(8) Fischer, M.
1900. winterhafer. Fühling's Landw. Ztg. 49: 718–723, 766–771, 806–810, illus.

(9) Garber, R. J.
1922. origin of false wild oats. Jour. Heredity 13: 40–48, illus.

(10) ——— and Quisenberry, K. S.
1923. delayed germination and the origin of false wild oats. Jour. Heredity 14: 267–274, illus.

(11) ——— and Quisenberry, K. S.
1927. natural crossing in oats at morgantown, west virginia. Jour. Amer. Soc. Agron. 19: 191–197.

(12) Griffee, F., and Hayes, H. K.
1925. natural crossing in oats. Jour. Amer. Soc. Agron. 17: 545–549.

(13) Haussknecht, C.
1885. über die abstammung des saathafers. Mitt. Geogr. Gesell. Jena 3: 231–242.

(14) Huskins, C. L.
1925. chromosomes in avena. Nature [London] 115: 677–678.

(15) ———
1926. genetical and cytological studies of the origin of false wild oats. Sci. Agr. 6: 303–313, illus.

(16) ———
1927. on the genetics and cytology of fatuoid or false wild oats. Jour. Genetics 18: [315]–364, illus.

(17) ———
1935. the origin and significance of fatuoids, speltoids, and other aberrant forms of oats and wheat. World's Grain Exhib. and Conf. Proc., Regina, Sask., 1933, v. 2, pp. 45–50.

(18) ——— and Fryer, J. R.
1925. the origin of false wild oats. Sci. Agr. 6: 1–13, illus.

(19) Jones, E. T.
1930. morphological and genetical studies of fatuoid and other aberrant grain-types in avena. Jour. Genetics 23: 1–68, illus.

(20) Körnicke, F., and Werner, H.
1885. handbuch des getreidebaues. 2 bd. Berlin.

(21) Marquand, C. V. B.
1922. varieties of oats in cultivation. Univ. Col. Wales, Welsh Plant Breed. Sta., Aberystwyth. [Bull.] ser. C, no. 2, 44 pp., illus.

(22) NILSSON-EHLE, H.
1907. OM HAFRESORTERS KONSTANS. Sveriges Utsädesför. Tidskr. 17: [227]–239, illus.
(23) ———
1911. ÜBER FÄLLE SPONTANEN WEGFALLENS EINES HEMMUNGSFAKTORS BEIM HAFER. Ztschr. Induktive Abstam. u. Vererbungslehre 5: 1–37, illus.
(24) ———
1921. FORTGESETZE UNTERSUCHUNGEN ÜBER FATUOIDMUTATIONEN BEIM HAFER. Hereditas 2: [401]–409.
(25) NISHIYAMA, I.
1931. THE GENETICS AND CYTOLOGY OF CERTAIN CEREALS. II. KARYOGENETIC STUDIES OF FATUOID OATS WITH SPECIAL REFERENCE TO THEIR ORIGIN. Japan. Jour. Genetics 7: 49–102, illus.
(26) STADLER, L. J., and KIRKPATRICK, R. T.
1930. COLUMBIA OATS, A NEW VARIETY FOR MISSOURI. Mo. Agr. Expt. Sta. Bull. 278, 12 pp., illus.
(27) STANTON, T. R., and COFFMAN, F. A.
1924. NATURAL CROSSING IN OATS AT AKRON, COLORADO. Jour. Amer. Soc. Agron. 16: 646–659.
(28) ——— COFFMAN, F. A., and WIEBE, G. A.
1926. FATUOID OR FALSE WILD FORMS IN FULGHUM AND OTHER OAT VARIETIES. Jour. Heredity 17: 152–165, 213–226, illus.
(29) TSCHERMAK, E.
1914. DIE VERWERTUNG DER BASTARDIERUNG FÜR PHYLOGENETISCHE FRAGEN IN DER GETREIDEGRUPPE. Ztschr. Pflanzenzücht. 2: [291]–312.
(30) ———
1918. BEOBACHTUNGEN BEI BASTARDIERUNG ZWISCHEN KULTURHAFER UND WILDHAFER. (AVENA FATUA). Ztschr. Pflanzenzücht. 6: 207–209.
(31) ———
1929. KULTUR- UND WILDHAFERBASTARDE UND IHRE BEZIEHUNGEN ZU DEN SOGENANNTEN FATUOIDEN. Ztschr. Induktive Abstam. u. Vererbungslehre 50: [450]–481 illus.
(32) ZADE, A.
1912. DIE ZWISCHENFORMEN VOM FLUGHAFER (AVENA FATUA) UND KULTURHAFER (AVENA SATIVA). Fühling's Landw. Ztg. 61: [369]–384.
(33) ———
1918. DER HAFER. EINE MONOGRAPHIE AUF WISSENSCHAFTLICHER UND PRAKTISCHER GRUNDLAGE. 335 pp., illus. Jena.

THE RELATIONSHIP OF THE PHOSPHATE CONCENTRATION OF SOLUTION CULTURES TO THE TYPE AND SIZE OF ROOT SYSTEMS AND THE TIME OF MATURITY OF CERTAIN PLANTS [1]

By ANNA L. SOMMER

Associate soil chemist, Department of Agronomy and Soils, Alabama Agricultural Experiment Station

INTRODUCTION

In an extended study of the nutrition of plants, a number of observations and studies may be made, definite results can be obtained, and conclusions drawn on some of them long before the work is concluded. In such a study on phosphorus nutrition three phases have already given rather definite results and it is deemed well to report them before the project is concluded. They are as follows: (1) The interrelationship between the size and type of root system, the phosphate concentration, and the amount of growth; (2) the size of root system as affected by the concentration of phosphate in the nutrient medium; and (3) the effect of different phosphate concentrations on the time of maturity.

For the purpose of these investigations plants of various kinds were grown in a series of tanks containing solutions with phosphate concentrations maintained at various constant levels. These cultures demonstrated that, all other conditions being the same, the minimum concentration for satisfactory growth varied with the kind of plant. The reason for such a difference may be either that one kind of plant may require a greater phosphate concentration in its nutrient media because of a difference in the amount necessary for physiological activities, or there may be a difference in the rate at which the root systems absorb the necessary ion, or both. Observations indicated that the type and extent of the root system might be an important factor. Experiments, therefore, were carried out to determine whether a relatively high concentration of phosphate was not often necessary, on account of the small extent of surface exposed, to supply the ion at a rate sufficiently rapid for satisfactory growth.

Observations on the root systems of the plants grown in these solutions indicated that higher phosphate concentrations did not stimulate root development, that is, did not cause a greater percentage increase for the roots than for the tops. Most studies in the past on the effect of phosphate on root systems took into consideration only increases in root weight and did not compare these increases with those of the tops. If the relative increase of the tops is as great as, or greater than that of the roots, a considerable part of the increase in the size of the roots of plants grown at higher phosphate concentrations may be due to the greater amount of food synthesized. Studies were therefore made in which the root-top ratio (weight) instead of absolute weights was taken as the criterion to determine whether or not phosphate actually stimulated root development.

[1] Received for publication June 5, 1935; issued March 1936.

Observations that a number of plants in solutions of low phosphate concentrations matured earlier than those in solutions of higher phosphate concentration led to a study of the effect of phosphate concentration on the time of maturity. The results of the study are reported herein.

PROCEDURE

The following plants were used in the experiments: Peas, buckwheat, corn, tomatoes, wheat, and cotton.

All plants were grown in solutions in 1,000-liter galvanized-iron tanks. These tanks were 4 feet in diameter and were coated on the inside with asphalt paint. The original composition of the solutions was the same for all constituents excepting monobasic potassium phosphate. The initial solution was made up as follows:

Salts:	*Grams per 1,000 liters of tap water*
KNO_3	400
$CaSO_4.2H_2O$	250
$MgSO_4.7H_2O$	150
$MnSO_4.2H_2O$	3.5
H_3BO_3	3.5

Phosphate determinations on samples filtered through hardened filter paper were made at least once daily, and PO_4 as KH_2PO_4 was added to maintain the desired concentrations, varying in these experiments from 0 to 12.8 p. p. m. A continuous current of air was used to agitate the solutions so that the PO_4 concentration would be uniform throughout the container. Twenty-five grams of heptahydrate ferrous sulphate ($FeSO_4.7H_2O$) were added (usually once a week) throughout the experiments. It was sometimes necessary during the early stages of growth to add the iron a little more frequently to maintain a good green color of the plants. The current of air was discontinued after the addition of the iron for a period of about 20 hours to prevent the rapid oxidation of ferrous to ferric iron. The iron precipitated the PO_4 almost completely so that during a period of 24 hours there was practically no PO_4 in solution. The current of air was started a few hours before the PO_4 was again added to oxidize the remaining ferrous iron to the ferric form so that the difference in the solubility of ferrous and ferric phosphate would not be a factor with which to reckon when restoring the PO_4 concentration. Since much of the iron was precipitated as the oxide and the solution tended to become acid, 25 grams of calcium carbonate were added when aeration was resumed. The addition of the calcium carbonate also helped in the ease with which the PO_4 concentration was reestablished by aiding in the precipitation of the iron. There was always undissolved calcium carbonate at the bottom of the tanks.

An excess of all nutrients excepting PO_4 was maintained. In most cases the plants were moved in rotation to the various tanks. This was done weekly just before the PO_4 concentration was restored. In other cases nutrient salts were added to the cultures that had grown sufficiently large to cause considerable decrease in the concentration of these salts.

The plants were supported by corks (one plant per cork) which fitted in holes in the tops of the containers.

EXPERIMENTAL RESULTS AND DISCUSSION

THE INTERRELATION BETWEEN THE SIZE AND TYPE OF ROOT SYSTEM, THE PHOSPHATE CONCENTRATION, AND THE AMOUNT OF GROWTH

Two types of experiments were carried out in the study of the relationship of the size and type of root system to the minimum PO_4 concentration necessary for satisfactory growth. In both types different kinds of plants were grown in the same containers in which the PO_4 concentrations of the solutions were maintained at various levels. In the first type a study was made of the relative size of the surface of the roots as compared with the growth of the plants. In the second type the PO_4 concentration of the expressed juice of the stems between the roots and the cotyledons was determined to ascertain whether it, and therefore the rate of supply of phosphorus to the plant, was proportional to the surface of the roots exposed to the culture solution.

Eight plants each of wheat, peas, buckwheat, corn, tomatoes, and cotton were transferred to each of four containers. The phosphate concentration in two of the tanks was maintained at 0.1 p. p. m. and in the other two at 0.8 p. p. m. These concentrations were chosen because previous experiments had shown that none of these plants made maximum growth in solutions in which the phosphate concentration was maintained at 0.1 p. p. m. but showed great differences at this concentration, and because all plants made good growth at 0.8 p. p. m. although some of them did not make maximum growth. The seeds of all plants were germinated at the same time, and all seedlings excepting the tomatoes were transferred to the culture solutions on the same day. The tomato plants were too small at that time and were therefore not set out until 3 days later.

Since there is no accurate way of measuring the surface of roots, all comparisons were a matter of judgment and photographs are the only evidence presented.

Photographs were taken after the plants had been growing in solutions 23 days. In order to show their structure, the plants were placed on a tray under water while they were being photographed. The roots appear dark in the photographs because of the ferric oxide deposited on them. The ferric oxide precipitate greatly increased the visibility of the root hairs; and where they are numerous, they appear on the photographs as dark patches with hazy outlines. The roots of plants grown in solutions of low PO_4 concentration (0.1 p. p. m.) are shown in figure 1. Figures 2, 3, and 4, show the comparative size of plants grown in solutions with PO_4 concentrations of 0.1 and 0.8 p. p. m.

The root-top ratio (apparent surface of the roots as compared with the size of the tops) arranged in descending order for the plants grown in the solutions having a PO_4 concentration of 0.1 p. p. m. is as follows: Wheat, buckwheat, peas, tomatoes, corn, and cotton. For a concentration of 0.8 p. p. m., the order was the same, with the exception of the tomato plants which grew the least. Root hairs on the wheat and buckwheat were by far the most numerous, those on tomatoes came next, thus making the order of size of root surface of these plants as compared with that of the peas rather doubtful since the peas had very few root hairs. No root hairs were found on the corn and cotton.

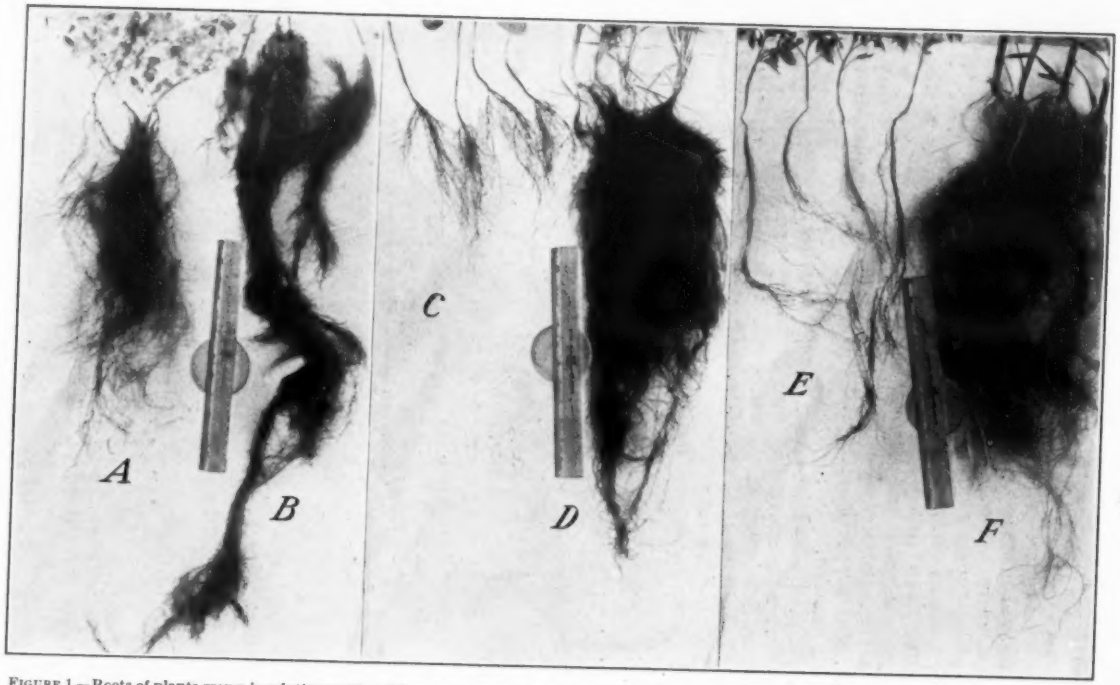

FIGURE 1.—Roots of plants grown in solutions with a PO_4 concentration of 0.1 p. p. m.: *A*, Peas; *B*, buckwheat; *C*, cotton; *D*, wheat; *E*, tomatoes, *F*, corn.

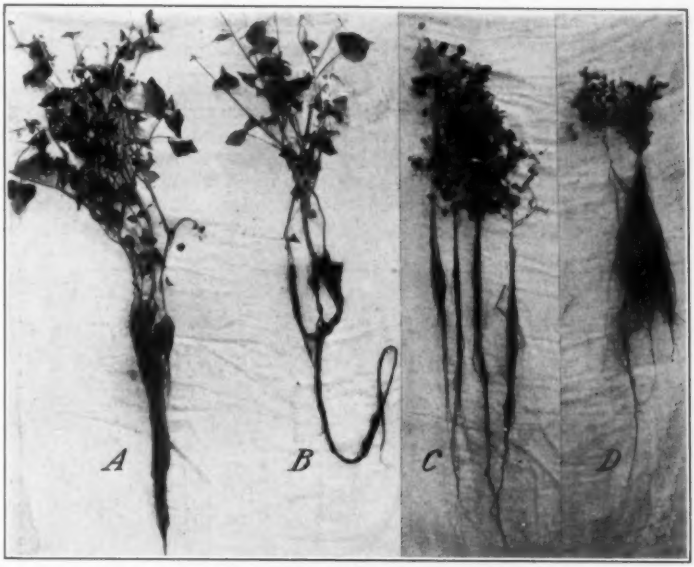

FIGURE 2.—Buckwheat (*A* and *B*) and pea plants (*C* and *D*) grown in solutions with PO_4 concentrations of 0.8 (*A* and *C*) and 0.1 p. p. m. (*B* and *D*).

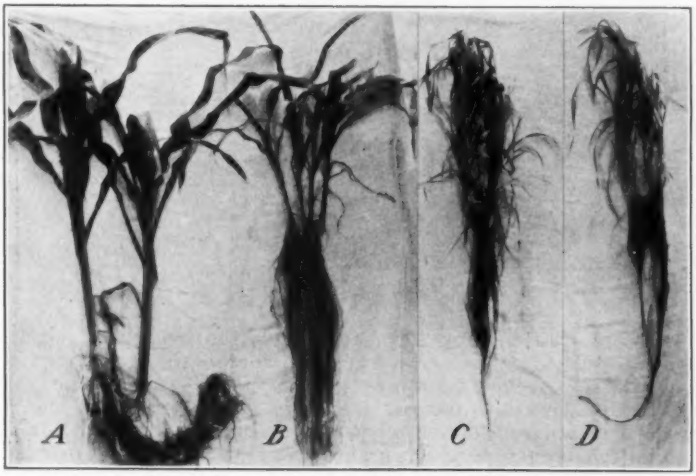

FIGURE 3.—Corn (*A* and *B*) and wheat plants (*C* and *D*) grown in solutions with PO_4 concentrations of 0.8 (*A* and *C*) and 0.1 p. p. m. (*B* and *D*).

One group of four tomato plants became shaded by the corn during the latter part of the experiment. These plants then began to make rapid growth and when harvested were much larger than the other tomato plants in the same container and those in the other container of the same phosphate concentration.

The difference in growth of different kinds of plants grown in the same kind of solution with varying PO_4 concentrations is shown in figure 5 and in table 5. The plants shown in figure 5 were transferred to the experimental solution in the cotyledon stage at the same time. A PO_4 concentration of 0.2 p. p. m. was maintained.

In the second type of experiments, cotton, buckwheat, and tomato plants were used in the studies of the relative rate at which the roots

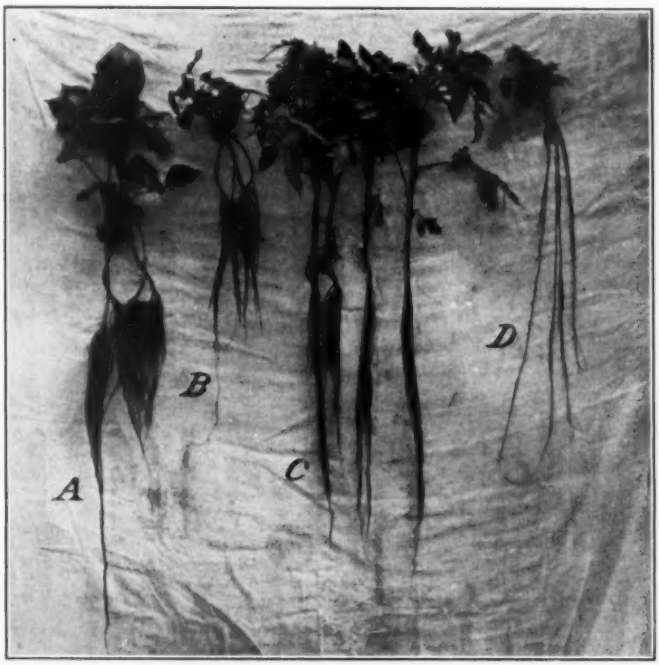

FIGURE 4.—Cotton (*A* and *B*) and tomato plants (*C* and *D*) grown in solutions with PO_4 concentrations of 0.1 (*B* and *D*) and 0.8 p. p. m. (*A* and *C*).

of different plants supply PO_4, because cotton requires a relatively high PO_4 concentration for good growth, whereas the other two plants grow well at much lower concentrations, and because these plants could be divided into comparable parts for analyses. In the first experiment two crops of buckwheat were grown in the same containers with cotton plants on which a study was being made to determine the minimum PO_4 concentration necessary for maximum growth. Earlier experiments indicated that good growth of cotton could not be obtained at concentrations below 0.8 p. p. m. This was therefore the lowest concentration used in this experiment. Solutions of two

other PO_4 concentrations, 3.2 and 12.8 p. p. m., were included. After the cotton had been growing in the containers for 28 days, the first crop of buckwheat was set out (in the cotyledon stage) and allowed to grow for 10 days when it was harvested and a second lot (also in the cotyledon stage) was placed in the solutions for the same length of time. At each buckwheat harvest some of the cotton plants were also harvested. The stems of both kinds of plants between the roots and

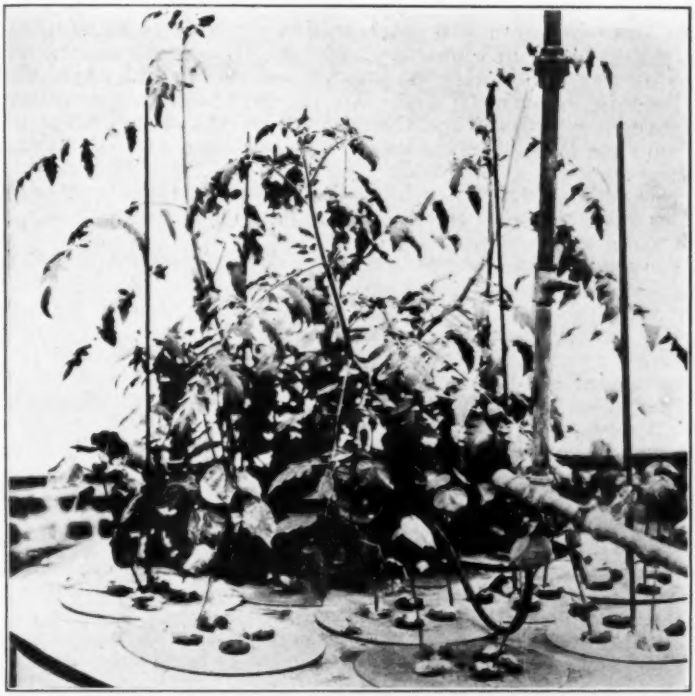

FIGURE 5.—Three tomato plants and cotton plants of the same age grown in solutions with a PO_4 concentration of 0.2 p. p. m.

cotyledons were frozen for phosphorus determinations of the expressed juice, and the young leaves were dried and analyzed for phosphorus.

In a second experiment PO_4 concentrations were maintained at 0.2, 0.4, 0.8, and 3.2 p. p. m. These concentrations included those at which very little growth of cotton was made and above which greater growth was not obtained. A crop of buckwheat was grown with the cotton as in the preceding experiment and when harvested tomato plants were substituted. The tomato plants were allowed to grow for 25 days when they were harvested with the remaining cotton plants. At this time there were not enough cotton plants left in solution of 0.2 p. p. m. for analyses and the tomato plants growing in solutions of higher PO_4 concentrations were so shaded by the rapidly growing cotton plants that they were discarded. Analyses were made as in the preceding experiment.

In a third experiment cotton and tomato plants were germinated and transferred to the containers at the same time. Phosphate concentrations of 0.2 and 3.2 p. p. m. were maintained. Some of both kinds of plants were harvested for analyses at the end of 21, 29, and 46 days.

There was considerable difference in the amount of growth made by the tomato plants at low PO_4 concentrations in the last two experiments. The weather was cloudy most of the time during the growth of the plants in the second experiment, and the tomato plants in the solutions with a low PO_4 concentration appeared to make as rapid growth as those in solutions of higher PO_4 concentrations. Clear weather prevailed during the first few weeks of growth of the plants of the third experiment; the tomato plants at low PO_4 concentrations made poor growth and had the deep purple color that develops when phosphorus is deficient. Cloudy weather began 12 days after the second harvest. The tomato plants in solutions with a phosphate concentration of 0.2 p. p. m. immediately began to make rapid growth, water of guttation appeared the morning after each cloudy day, and the new growth was not purple.

The results of these experiments are shown in table 1. With one exception the amount of phosphorus in the expressed juice of the buckwheat stems was considerably greater than that of the cotton stems. In this exceptional case the buckwheat for the last days was shaded by the rapidly growing cotton. Where the tomato plants were making good growth, in solutions of both high and low PO_4 concentration, the results were similar to those of the buckwheat but those tomato plants that were making poor growth at low PO_4 concentrations had less PO_4 in the expressed juice of the stems than did the cotton. Where the amount of phosphorus in the expressed juice of the stems was less for the tomato plants than for the cotton, the plants had been transferred to the culture solutions and had grown in sunny weather. Plants of the same group which were harvested after 46 days and which were harvested after a number of cloudy days contained more phosphorus than did the cotton harvested at the same time.

There appears to be some factor which, under conditions of bright sunlight and low PO_4 concentration, decreases phosphate absorption for the tomato plant. Where the light intensity was sufficiently low the amount of growth of the tomato plant was that which would be expected for a plant with such a root system at low PO_4 concentrations.

Further evidence of the difference in the PO_4 concentration necessary for the good growth of tomato plants under different light conditions was obtained in another experiment. One group of plants grown for the first few weeks in cloudy weather in a solution with a PO_4 concentration of 0.1 p. p. m. made better growth than did plants grown during sunny weather in solutions with a PO_4 concentration of 0.2 p. p. m. Varying weather conditions appeared to cause different rates of growth for the plants growing in solutions with a PO_4 concentration of 0.1 p. p. m. After the first few weeks of cloudy weather there were about 2 weeks in which sunny weather predominated. The tomato plants appeared to stop growing, and a purple coloration began to appear. After a day or two of cloudy weather, water of guttation again appeared and growth was resumed.

TABLE 1.—*Phosphorus in the expressed juice of stems and in young leaves of cotton, buckwheat, and tomato plants grown in cultures of various phosphate concentrations*

PO_4 concentration in parts per million and experiment no.[1]	Phosphorus in expressed juice of stems			Phosphorus in young leaves (dry weight)					
				Total			Soluble		
	Cotton	Buckwheat	Tomato	Cotton	Buckwheat	Tomato	Cotton	Buckwheat	Tomato
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0.2:									
3	0.0035	0.019	.....	0.40	0.80	.....	0.15	0.58	.....
5	.0033	.....	0.0021	.44	.....	0.42	.065	.....	0.057
5′	.0046	.....	.0025	.45	.....	.44	.....	.....	.18
5″	.0030	.....	.0042	.25	.....	.65	.14	.....	.22
0.4:									
3	.0055	.029	.....	.58	.87	.....	.39	.57	.....
4	.0065	.....	.0183	.66	.....	.16	.40	.....	.89
0.8:									
1	.0090	.020	.....	.55	.76	.....	.35	.53	.....
2	.0090	.030	.....	.76	1.10	.....	.52	.86	.....
3	.0089	.024	.....	.72	1.00	.....	.48	.57	.....
3.2:									
1	.0095	.034	.....	.68	.86	.....	.46	.65	.....
2	.020	.040	.....	1.18	.88	.....	.84	.61	.....
3	.057	.028	.....	.99	.87	.....	.52	.52	.....
5	.0072	.....	.017	.68	.....	1.09	.33	.....	.82
5′	.0073	.....	.021	.70	.....	1.05	.41	.....	.90
5″	.011	.....	.049	.93	.....	1.12	.27	.....	.83
12.8:									
1	.012	.36	.....	1.00	.90	.....	.68	.....	.67
2	.028	.57	.....	.99	.88	.....	.80	.....	.58

[1] 1 and 2, first experiment of test; cotton plants set out at the same time but different lots of buckwheat. 3 and 4, second experiment; cotton set out at the same time; first buckwheat was set out with cotton 3, and later replaced by tomatoes 4. 5, 5′, and 5″, third experiment; cotton and tomato plants all set out at the same time but harvested at 3 different times.

A number of investigators have shown that plants differ greatly in their capacity to use the phosphorus of the soil. Hartwell (*3, p. 7*)[2] found that carrots secured their entire needs under conditions where turnips were practically unable to grow without phosphatic application; millet and tomatoes ranked next to carrots; and beets and rape next to turnips. Cotton requires phosphorus fertilization whereas oats will make good growth without it. The soil is so complex a system that very little has been learned from soil studies to explain these differences. With solution cultures many of the difficulties are removed, and the problem may be reduced to one of the PO_4 concentration necessary for the normal growth of the plant under certain conditions.

Both water culture and field experiments have shown that wheat and buckwheat are able to grow with lower PO_4 concentration than some other plants. Both of these plants produce an enormous number of root hairs and, therefore, have a great root surface. If judged by dry weight, buckwheat would have a very small root system, but its fine roots and numerous root hairs with little weight present a very large surface for absorption. In solution cultures tomato plants vary greatly in the number of root hairs produced, but in all experiments here have produced very large root systems with many fine roots. The minimum PO_4 requirement for tomato plants appears to vary markedly with the amount of light; that is, the greater the light intensity the higher the PO_4 concentration necessary.

[2] Reference is made by number (italic) to Literature Cited, p. 147.

Cotton had the poorest root system of the plants studied. There were no root hairs or very fine roots on plants grown in the culture solutions. Its growth at low PO_4 concentrations was also much the poorest, with the exception of tomatoes grown in bright light. In cloudy weather, at 0.2 p. p. m., it made very poor growth, while tomato plants in the same containers were making very rapid growth.

The requirements of greater amounts of phosphate for some plants than for others under field conditions can be explained only in part by solution-culture experiments. Snow (*5*) found that corn produced no root hairs in solution cultures but that they were numerous under normal soil conditions. This may explain the findings of Tidmore (*6*, *7*) that certain soils producing good corn without PO_4 fertilization had in their displaced solutions PO_4 concentrations of only 0.03 to 0.05 p. p. m., while solution cultures with a PO_4 concentration of 0.05 p. p. m. produced very poor plants. The growth of corn obtained in solutions with a PO_4 concentration of 0.1 p. p. m. might be expected for such a root system. According to weight, corn in solution cultures would be considered to have a large root system, but the roots for the most part are coarse and therefore the relative surface exposed is small. Another factor probably important, especially in neutral and alkaline soils, is the relative amount of carbon dioxide (CO_2) given off by different plants. The carbon dioxide under these conditions may serve two functions in phosphorus nutrition, that of making more soluble the insoluble phosphates, and of producing in the immediate vicinity of the root a reaction more favorable for the absorption of anions. Newton (*4*) found that peas absorbed more calcium than did barley when grown in soil. He found that the roots of peas produced more carbon dioxide than did those of barley and explained the greater absorption of calcium by the peas on the assumption that the solubility of the calcium was increased by the carbon dioxide, thus making more calcium available for the peas than for the barley. Breazeale and McGeorge (*1*) say that carbon dioxide is the most important single factor in the fertility of alkaline soils. In many cases, because of the effect of carbon dioxide, the displaced soil solution may not give a true picture of what is available for the root. It is probably impossible, moreover, to get an adequate picture of conditions where root hairs are in contact with the colloidal fraction of the soil.

THE SIZE OF THE ROOT SYSTEM AS AFFECTED BY THE CONCENTRATION OF PHOSPHORUS IN THE CULTURE SOLUTIONS

In this study of the effect of PO_4 concentration on the size of the root system, particular attention was paid to the weight of the roots as compared with that of the tops, since it seems valid to say that phosphate fertilization stimulates root development only in case the relative increase in the weight of the roots is greater than that of the tops.

Plants were grown under two sets of conditions. In one experiment tomato, pea, cotton, wheat, and corn plants were grown in solutions of which the PO_4 concentration was maintained at various low levels. In the other, tomato, wheat, corn, and cotton plants were first grown for several weeks with an adequate supply of PO_4; some of the plants of each kind were transferred to phosphate-free solutions, while the rest were allowed to continue their development in solutions with a PO_4 concentration sufficiently high for good growth.

TABLE 2.—*Root-top ratio, by weight, of plants of four kinds when grown in culture solutions of various phosphate concentrations*

Kind of plant and experiment no.[1]	Root-top ratio when grown at PO_4 concentration of—						Comments
	0.1 p. p. m.	0.2 p. p. m.	0.4 p. p. m.	0.8 p. p. m.	3.2 p. p. m.	12.8 p. p. m.	
Tomato:							
1		0.50		0.33	0.28		Young plants.
2		.47			.39		Do.
3		[2].37	[2]0.15				Do.
4	[2]2.55				[2].24		Do.
5		.54			.29		Do.
Pea:							
1		.06		.04	.04		Mature plants.
Cotton:							
1				.13	.05	0.05	Do.
2		[2].92	[2].77	[2].49	[2].32		Young plants.
3			[2].44	[2].28	[2].23		Rapidly growing plants, same as above but 1 month older.
4		[2]1.35			[2].48		Between 5 and 6 weeks old.
5		.19	.08	.08	.12		Plants at 0.2, 0.4, and 0.8 p. p. m. had finished growing; plants at 3.2 p. p. m. were same age but still growing.
6		[2].82			[2].41		Young plants.
Wheat:							
1		.04		.10	.07		Plants all the same age, but those at 0.2 p. p. m. had stopped growing and produced ripe seed; those at 0.8 and 3.2 p. p. m. still making vegetative growth.
Corn:							
1	.78	.55			.19		Young plants.

[1] Nos. 1, 2, etc., correspond to those numbers of table 3 and refer to the same plants.
[2] Green weights; all of the others, dry weights.

TABLE 3.—*Weights of roots and tops of plants of four kinds when grown in culture solutions of various phosphate concentrations*

Kind of plant and experiment no.[1]	Weight of roots and tops when grown at PO_4 concentration of—											
	0.1 p. p. m.		0.2 p. p. m.		0.4 p. p. m.		0.8 p. p. m.		3.2 p. p. m.		12.8 p. p. m.	
	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops
Tomato:	*G.*	*G.*	*G.*	*G.*	*G.*	*G.*	*G.*	*G.*	*G.*	*G.*	*G.*	*G.*
1			4.9	9.9			9.8	29.9	10.7	38.0		
2			[2]2.6	[2]5.5					11.8	30.5		
3			[2]1.8	[2]4.9	[2]1.5	[2]9.7						
4	[2]2.8	[2]1.1							[2]25.1	[2]105.0		
5			[2]26.5	[2]48.9					[2]143.5	[2]490.5		
Pea: 1			.6	10.1			1.8	40.1	1.8	47.6		
Cotton:												
1							16.2	129.5	11.5	219.5	10.1	196.6
2			[2]2.3	[2]2.5	[2]4.2	[2]5.5	[2]8.4	[2]17.1	[2]9.4	[2]29.1		
3					[2]58.0	[2]136.0	[2]62.0	[2]224.0	[2]74.0	[2]327.0		
4			[2]3.8	[2]2.8					[2]31.3	[2]64.6		
5			.6	3.2	4.3	54.0	24.4	320.0	62.9	510.0		
6					[2]1.4	[2]1.7			[2]2.1	[2]5.1		
Wheat: 1			4.0	92.8			6.7	69.4	5.9	89.0		
Corn: 1	1.4	1.8	2.7	4.9					3.5	18.8		

[1] Nos. 1, 2, etc., correspond to those numbers of table 2 and refer to the same plants.
[2] Green weights; all of the others dry weights.

Where plants were grown in solutions of various PO_4 concentrations the root-top ratio decreased with increasing PO_4 concentrations in all cases excepting one wheat and one cotton series, which will be discussed later (table 2). Not only was the root-top ratio usually greater at lower PO_4 concentrations, but the average weight of the roots in some cases (tomatoes (3), cotton (1), and wheat (1), table 3) was greater for some in the lower than in the higher concentrations. The much greater growth of the tops of cotton plants as compared with the roots for increasing PO_4 concentrations is shown in figures 6 and 7.

These results are just the opposite of those reported by Turner (*8*) on wheat, barley, and cotton. His solutions, however, were not comparable to those used in this experiment since his low phosphate concentration was not only higher than the highest used in the experiment described above but higher than is commonly found in soil solu-

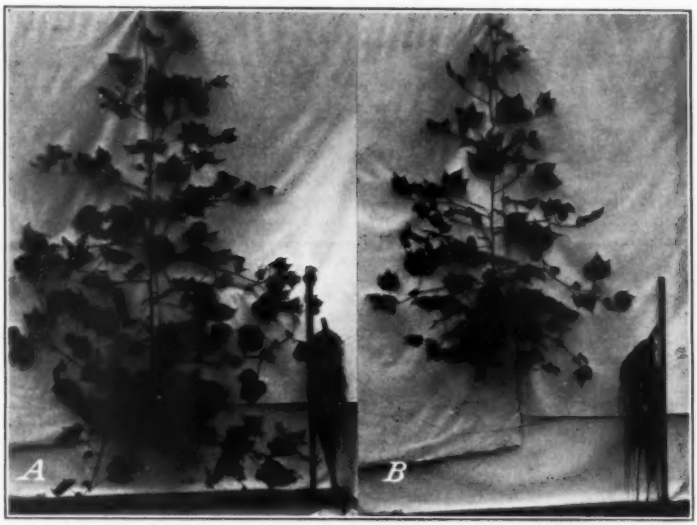

FIGURE 6.—Cotton plants grown in solution with PO_4 concentrations of 3.2 (*A*) and 0.8 p. p. m. (*B*).

tions. The total concentration of salts in his low PO_4 solution, moreover, was only a little over half that of his high PO_4 solution.

The root-top ratio of wheat plants grown at the lowest PO_4 concentration and that of cotton at the highest concentration in one series appeared to be exceptions to the rule that the root-top ratios decreased with increasing PO_4 concentration. The wheat plants at this low concentration had finished growing and produced ripe seeds, while those at the two higher concentrations were still producing many new tillers and making rapid vegetative growth. The exceptional cotton plants were making vegetative growth when harvested, while those at the three lower concentrations had apparently stopped growing. A comparison of plants at different stages of growth is probably misleading since, as is shown by the work of Brenchley and Jackson (*2*), there is, in general, a decrease in the root-top ratio with the age of the plant.

Plants transferred from solutions of adequate PO_4 concentrations to phosphate-free solutions showed marked increases in root-top ratios. With one exception the amount of phosphate in the solution before the plants were transferred was 1.0 p. p. m. or less, so that the relatively large amount of growth of the roots as compared with that of the tops after transference to phosphate-free solutions could hardly be due to phosphorus stored in the roots. In two cases (wheat (1) and corn (1), table 4) the average weight of the roots of plants transferred to solutions without phosphate was greater than that of the plants allowed to remain in solutions containing phosphate. The results of this group of experiments are shown in table 4.

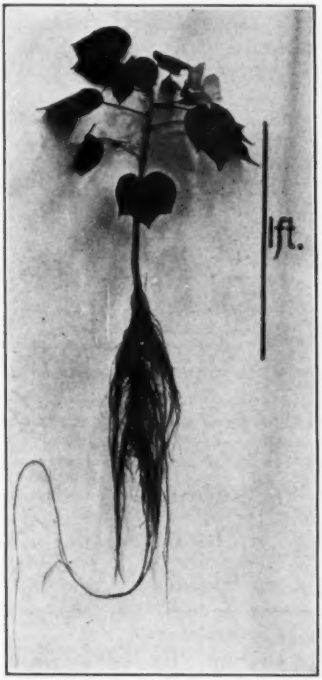

FIGURE 7.—Cotton plant grown in solution with a PO_4 concentration of 0.2 p. p. m.

THE EFFECT OF DIFFERENT PHOSPHATE CONCENTRATIONS ON THE TIME OF MATURITY

Peas, cotton, and wheat were grown in solutions with phosphate concentrations maintained at various levels and observations made as to the time of their maturity. The results of this work (table 5) show that with the exception of cotton at the lowest concentration, at which there was very little growth and no seed matured, the plants at the lower concentrations matured the earliest.

It is commonly accepted that large applications of phosphorus hasten maturity of crops in countries where the growing period is short. Experiments with solution cultures, both as reported in this paper and observations made at other times, have shown that the growing period of plants is shortened where the amount of PO_4 is low. In the culture solutions all other necessary elements were maintained in adequate amounts so that phosphorus was the only limiting factor. Since under conditions of low fertility in the field unfertilized plants mature earlier than those with adequate fertilization, it seems probable that the depletion of one or more of several of the necessary elements may be responsible for this early maturity. Where maturity is hastened by large applications of phosphorus the effect is therefore probably an indirect one. That is, there is greater growth because of the large applications of PO_4 and, owing to its more rapid growth, the plant absorbs more rapidly other ions and thus some other element, perhaps nitrogen, becomes a limiting factor causing early maturity.

50929—36——5

TABLE 4.—*Average weight of tops and roots and root-top ratio of plants of 4 different kinds when grown in culture solutions of various phosphate concentrations and later transferred to solutions containing and not containing phosphate*

Kind of plant and experiment no.	Time with PO_4 before transference	PO_4 concentration of culture	After transference to solution—							
			Containing no PO_4				Containing PO_4			
			Weight of roots	Weight of tops	Root-top ratio	Time	Weight of roots	Weight of tops	Root-top ratio	PO_4 concentration of culture
Tomato:	*Weeks*	*P. p. m.*	*Grams*	*Grams*		*Weeks*	*Grams*	*Grams*		*P. p. m.*
1	7	0.4	0.6	1.5	0.40	3	1.1	5.5	0.20	0.4
2	7	.4	1.5	2.7	.55	6	10.7	47.0	.23	.4
3	7	.4	2.1	4.5	.47	6	23.0	77.1	.30	.4
4	7	.4	[1] 12.0	[1] 16.0	[1] .75	3	[1] 21.7	[1] 73.5	[1] .30	.4
5	7	.4	[1] 19.5	[1] 21.5	[1] .91	.6	[1] 201.2	[1] 647.0	[1] .31	.4
6	4	[2] 1.0	[1] 6.7	[1] 11.6	[1] .58	2	[1] 21.8	[1] 40.1	[1] .54	[2] 1.0
7	4	[2] 1.0	[1] 11.4	[1] 14.8	[1] .77	4	[1] 48.1	[1] 146.1	[1] .33	[1] 1.0
Wheat:										
1	9	.4	1.8	3.1	.58	3	1.1	7.6	.14	.4
2	9	.4	2.6	5.0	.52	6	5.0	22.1	.23	.4
3	9	.4	[1] 22.4	[1] 16.0	[1] 1.40	3	[1] 33.0	[1] 67.0	[1] .49	.4
4	9	.4	[1] 26.2	[1] 18.2	[1] 1.44	6	[1] 75.4	[1] 186.4	[1] .40	.4
5	4	.4	[1] 9.0	[1] 4.7	[1] 1.91	4	[1] 11.6	[1] 19.0	[1] .61	.4
Corn: [3]	4	.4	5.2	15.08	.34	6	4.4	23.2	.19	.4
Cotton: [3]										
1	9	1,040	3.1	27.4	.11	6	6.5	69.0	.09	50
2	6	1,040	3.2	25.1	.13	9	6.5	69.0	.09	50

[1] Green weight; all others dry weight.
[2] PO_4 concentration brought up to 1.0 p. p. m. once a week.
[3] Data from an experiment to determine whether cotton, if given large amounts of phosphate during earlier stages of growth, could make normal growth afterward if deprived of phosphate in the culture media.

TABLE 5.—*The effect of various phosphate concentrations in the culture solutions on the time of maturity of plants of four different kinds*

[The seeds of all plants of each group were germinated and the plants transferred to the experimental solutions and harvested at the same time]

Plant	PO_4 concentration	Average dry weight per plant	Description of plant at time of harvest	Comments
	P. p. m.	*Grams*		
Pea	0.2	10.7	Dead 1 week; seed mature.	
	.8	41.9	Drying at bottom; some of seed still immature.	
	3.2	47.6	....do....	A little less dry than plants at 0.8 p. p. m.
Wheat	.2	96.4	Beginning to dry; seed mature.	Well-filled, mature seeds probably account for the high dry weight as compared with the larger plants at higher PO_4 concentrations.
	.8	76.1	Making rapid growth; many new tillers; heads at all states of growth.	
	3.2	94.9	....do....	Plants injured by aphids when first heads appeared.
Cotton	.8	145	Not growing.	Plants of this group were set out Aug. 10 and harvested Jan. 2; all had many well-developed bolls, none of which opened, probably due to continued cloudy weather during November and December.
	3.2	231	Making good growth and blooming.	
	12.8	199	....do....	
	.2	4	Some green leaves but making no growth.	Four small bolls to 41 plants; bolls did not open.
	.4	54	All leaves off; 96 percent of bolls open.	Average number of bolls per plant, 6.
	.8	320	Most of leaves off; 90 percent of bolls open.	Average number of bolls per plant, 41.
	3.2	510	Some plants still making good growth; 49 percent of bolls open.	Average number of bolls per plant, 74.

SUMMARY AND CONCLUSIONS

Solution-culture experiments were carried out in a study of the relationship of the PO_4 concentration of the medium to the type and size of the root system and the time of maturity of certain plants.

Two types of experiments were carried out in an effort to determine whether or not the size and type of root system played a part in limiting the minimum PO_4 concentration at which certain kinds of plants could make satisfactory growth. In one type of experiment different kinds of plants were grown in solutions with PO_4 concentrations maintained at 0.1 and 0.8 p. p. m., and a study was made of the relationship of the size and type of root system to the amount of growth. In another type of experiment, cotton, a plant with a root system presenting comparatively small surface for absorption and requiring a relatively high PO_4 concentration, was grown in containers with buckwheat and tomatoes, plants having more extensive root systems and making better growth at low PO_4 concentrations. Phosphate concentrations in the solutions were maintained at various levels, and the amount of phosphorus in the part of the stem between the roots and cotyledons and in the leaves was determined. Plants with the larger root systems made better growth at low PO_4 concentrations and more phosphorus was found in their stems than in the stems of plants with root systems of comparatively small surface.

Two groups of experiments were carried out in a study of the so-called stimulating effect of phosphorus on root development. In one group, plants were grown in solutions in which the PO_4 concentrations were maintained at various low levels; in the other group plants were first grown with an adequate supply of phosphorus and then transferred to phosphate-free solutions. Comparisons showed that increasing PO_4 concentrations resulted in decreases of the root-top ratios.

The effect of PO_4 concentrations on the time of maturity was studied by growing different kinds of plants in solutions in which the PO_4 concentrations were maintained at various levels. Plants in lower PO_4 concentrations matured the earliest.

Size and type of root system probably play an important part in determining the minimum PO_4 concentration at which the different kinds of plants make satisfactory growth.

High concentrations of PO_4 in solution cultures do not stimulate root development.

Plants growing in culture solutions of low PO_4 concentrations mature earlier than those grown in solutions of high PO_4 concentration.

LITERATURE CITED

(1) Breazeale, J. F., and McGeorge, W. T.
1932. Nutritional disorders in alkaline soils as caused by deficiency of carbon dioxide. Ariz. Expt. Sta. Tech. Bull. 41, pp. [113]–153, illus.

(2) Brenchley, W. E., and Jackson, V. G.
1921. Root development in barley and wheat under different conditions of growth. Ann. Bot. [London] 35: 533–556, illus.

(3) Hartwell, B. L.
1919. Thirty-first annual report . . . R. I. Agr. Expt. Sta. Ann. Rept. (1918) 31, 8 pp.

(4) NEWTON, J. C.
1923. A COMPARISON OF THE ABSORPTION OF INORGANIC ELEMENTS, AND OF THE BUFFER SYSTEMS OF LEGUMES AND NON-LEGUMES, AND ITS BEARING UPON EXISTING THEORIES. Soil Sci. 15: 181–204, illus.
(5) SNOW, L. M.
1905. THE DEVELOPMENT OF ROOT HAIRS. Bot. Gaz. 40: 12–48, illus.
(6) TIDMORE, J. W.
1930. PHOSPHATE STUDIES IN SOLUTION CULTURES. Soil Sci. 30: 13–31, illus.
(7) ———
1930. THE PHOSPHORUS CONTENT OF THE SOIL SOLUTION AND ITS RELATION TO PLANT GROWTH. Jour. Amer. Soc. Agron. 22: 481–488.
(8) TURNER, T. W.
1929. EFFECT OF MINERAL NUTRIENTS UPON SEED PLANTS. II. PHOSPHATES. Bot. Gaz. 88: 85–95.

THE EFFECTS OF LOW-PHOSPHORUS RATIONS ON GROWING PIGS[1]

By C. E. AUBEL, *swine investigator, Department of Animal Husbandry*, J. S. HUGHES, *animal nutritionist, Department of Chemisty*, and H. F. LIENHARDT, *pathologist, Department of Veterinary Medicine, Kansas Agricultural Experiment Station*[2]

INTRODUCTION

The discovery that aphosphorosis in domestic animals occurs quite frequently under ordinary feeding conditions in many localities throughout the world, has served to direct the attention of nutrition workers to the role of phosphorus in animal nutrition. As a result, many studies have been made; in most of them cattle or sheep were used as experimental animals. Among such studies are those of Theiler (*9, 10*);[3] Malan, Green, and du Toit (*7*); and Eckles and his associates (*4, 5*). Considerable experimental work (*4, 8*) has dealt with the effect of phosphorus-deficient rations on animals and with the correction of these deficiencies. The literature reports rather fully the effects of low-phosphorus rations on other species of animals, but not on swine.

However, Bethke and his associates (*1*) have described the effects of the calcium-phosphorus relationship of the ration on the growth and bone formation in the pig, and Dunlop (*3*) has described the importance of calcium and phosphorus in the ration, as well as the relationship between these two elements with and without vitamin D.

Otherwise the influence of low-phosphorus rations on body growth and on the mineral composition of the blood and bones has been little discussed; and no specific information, so far as the authors are aware, has been recorded regarding other effects on growing pigs, of phosphorus-deficient rations in which the calcium and other essential nutrients were supplied in adequate amounts. The effects of low-phosphorus rations on growing pigs, observed during the progress of experiments designed primarily to show the phosphorus requirements for growing swine, are here presented. The results of two feeding tests are recorded in which young pigs were fed rations differing only in phosphorus content.

EXPERIMENTAL PROCEDURE

The basal ration employed in these experiments consisted of 74 percent of pearl hominy, 10 percent of tapioca roots, 10 percent of blood meal, 4 percent of alfalfa-leaf meal, 1.5 percent of dried brewers' yeast, 0.5 percent of iodized salt, and 5 cc of cod-liver oil per pig per day. Enough calcium carbonate was added to these ingredients to bring the calcium content up to 0.77 percent in the first experiment and up to 0.70 percent in the second.

[1] Received for publication July 9, 1935; issued March 1936. This paper represents a portion of a thesis presented by C. E. Aubel in partial fulfillment of the requirements for the degree of doctor of philosophy at the University of Minnesota in 1935. Contribution no. 114 of the department of animal husbandry, no. 190 of the department of chemistry, and no. 62 of the department of veterinary medicine.

[2] The authors wish to give credit to Prof. J. F. Merrill for making the chemical analyses of the feeds and to R. R. Roepke for the analyses of the blood. Credit is also due Prof. D. L. Mackintosh for assistance in slaughtering.

[3] Reference is made by number (italic) to Literature Cited, p. 159.

Three lots of six pigs each were used in each of the two experiments, which were carried on for 24 weeks each. The experiments were started shortly after the pigs were weaned at about 9 weeks of age, when they weighed from 40 to 50 pounds each. All the lots were housed in a well-lighted and well-ventilated building provided with individual pens paved with concrete and measuring 8 by 8 feet. Each pen had a 10- by 8-foot outside exercise area open to the south, paved with concrete and fenced with wire. This outside area allowed the pigs access to the direct rays of the sun and exposed them, to a considerable extent, to ultra-violet rays.

All the animals were fed individually twice a day. The amount fed daily was changed from time to time, but each received the amount of feed that the one consuming the least feed would eat morning and evening. This arrangement insured all the pigs ingesting the same amount of feed.

The pigs were weighed and measured every 28 days throughout the experiments. An analysis of the calcium and inorganic phosphorus in the blood was made at the beginning of each experiment and at the end of each 28 days. A single sample of blood was drawn from the tail for these determinations. The Youngburg and Youngburg (*11*) method was used for the determination of inorganic phosphorus, and the calcium was determined by the Clark and Collip (*2*) method. The progressive effects of the low-phosphorus rations on the development of the bones, carcasses, and internal organs were noted at the end of each 56 days, when two animals from each lot were slaughtered.

In order to feed the different lots of pigs at different levels of phosphorus, monocalcium phosphate was incorporated in the basal ration to bring the phosphorus content up to the desired level. In the first experiment the phosphorus content of the ration fed lot 1 was 0.15 percent, that of the ration fed lot 2, 0.29 percent, and that of the ration fed lot 3, 0.59 per cent. The experimental pigs were fed phosphorus at these levels for 7 weeks, at the end of which time a second mixture was made, which, because of variations in the phosphorus content of the ingredients, contained slightly higher amounts of phosphorus. The amounts were 0.18 percent in that fed to lot 1, 0.33 percent in that fed to lot 2, and 0.59 percent in that fed to lot 3. Rations containing phosphorus at these levels were fed during the remaining 17 weeks of the experiment.

In the second experiment the phosphorus content of the ration of lot 4 was 0.15 percent; that of the ration of lot 5, 0.23 percent; and that of the ration of lot 6, 0.30 percent. Enough feed containing these amounts of phosphorus to supply the pigs during the entire 24 weeks of feeding was mixed before the experiment began.

EXPERIMENTAL DATA

In the course of these experiments five very decided effects of feeding the pigs the low-phosphorus rations were observed. These were: (1) The effect on the appetite; (2) the effect on the utilization of feed and storage of energy; (3) the effect on growth of the body and the development of bone and muscle; (4) the effect on the inorganic phosphorus in the blood; (5) the effect on the consumption of water and the excretion of urine.

EFFECT ON THE APPETITE

Loss of appetite in the low-phosphorus pigs in lots 1 and 4 began to appear as early as the fifth week after the pigs were placed on feed. This made it necessary to feed very carefully in order to have them consume the same amounts of feed as the other pigs. By the end of the fifth month it was no longer possible to get these pigs to eat the ration in a dry form. It was possible, however, because of an increased thirst which they developed, to induce them to eat their feed by mixing it with the drinking water. Toward the end of the experiments it was necessary to withhold all drinking water except that mixed with the feed. By mixing small portions of the feed in the water five or six times each day, by the end of the experiments it was possible to induce the pigs to consume nearly 6 pounds of feed daily. So greatly did they crave water that they soon learned to wait for the feed to settle, and it was necessary for an attendant to keep stirring the mixture in order to have the feed consumed.

No difficulty over loss of appetite was experienced with the pigs in lots 2, 3, 5, and 6, which were on higher levels of phosphorus.

TABLE 1.—*Average feed consumed daily per pig, daily gains in weight, and feed required per 100 pounds gain in weight by 28-day periods in experiments 1 and 2*

EXPERIMENT 1

Period (28 days) no.	Pigs [1]	Feed consumed daily			Average daily gain in weight			Feed required per 100 pounds gain in weight		
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
	Number	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	6	2.5	2.5	2.5	0.72	0.81	0.85	347.2	308.6	294.1
2	6	3.1	3.1	3.1	.74	.96	.99	418.9	322.9	313.1
3	4	3.9	3.9	3.9	1.06	1.09	1.16	367.9	357.7	336.1
4	4	4.5	4.5	4.5	1.15	1.26	1.20	391.3	357.1	375.0
5	2	5.5	5.9	5.9	.74	1.41	1.31	743.2	418.4	450.3
6	2	5.7	5.9	5.9	1.28	1.50	1.48	445.3	393.3	398.6
Weighted average		3.7	3.7	3.7	.90	1.07	1.08	417.1	344.6	341.0

EXPERIMENT 2

		Lots 4, 5, and 6	Lot 4	Lot 5	Lot 6	Lot 4	Lot 5	Lot 6
	Number	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	6	2.7	0.65	0.83	0.91	415.3	325.3	296.7
2	6	3.3	.81	1.00	1.06	407.4	330.0	311.3
3	4	3.5	.86	1.05	1.19	406.9	333.3	294.1
4	4	4.4	.87	1.34	1.41	505.7	328.3	312.0
5	2	5.7	1.41	1.61	1.62	404.2	354.0	351.8
6	2	6.0	1.05	1.28	1.27	571.4	468.7	472.4
Weighted average		3.7	.85	1.09	1.16	439.1	342.6	321.7

[1] Of the original 6 pigs, 2 were slaughtered at the end of the second period, 2 at the end of the fourth, and 2 at the end of the sixth.

EFFECT ON THE UTILIZATION OF FEED AND THE STORAGE OF ENERGY

That the pigs on the low-phosphorus diet did not utilize their feed as well as those on the higher levels of phosphorus is shown in table 1. The data show that lots 1 and 4, the low-phosphorus groups, had during each period of the experiment the largest feed consumption

per 100 pounds gain. In lot 1, experiment 1, the consumption per 100 pounds gain fluctuated from period to period but was exceptionally high in period 5. As a rule, the fluctuation varied as the average daily gains, but the very high requirement of feed in period 5 was accompanied by a low average daily gain. No explanation can be offered for this unless the sudden change from a dry-feeding method to a thin-slop method might have caused some irregularity in body weight. In lot 4, experiment 2, in which the change was made gradually from dry to slop feeding when the pigs began to lose their appetites, no such fluctuation was observed.

While no direct measurements of the storage of energy were made in these experiments, on the basis of gains in weight it is evident that the pigs receiving the low-phosphorus ration stored less energy than those receiving higher amounts. The difference, however, is not so great as the difference in the body weights would indicate, because the low-phosphorus-fed pigs carried a higher percentage of fat and showed better finished carcasses.

The thickness of the back fat opposite the seventh, eleventh, and thirteenth dorsal vertebrae was measured in experiment 2. The results, shown in table 2, indicate, according to the fat index suggested by Hankins and Ellis (*6*), that there was an average of approximately 96 pounds of fat on the carcasses of the low-phosphorus pigs, as compared with 108 pounds on those of the high-phosphorus pigs in lots 5 and 6.

TABLE 2.—*Average thickness of the back fat at the seventh, eleventh, and thirteenth ribs of two pigs in lots 4, 5, and 6 at the end of the fourth and sixth periods of experiment 2*

Period (28 days) no.	Thickness at seventh vertebra			Thickness at eleventh vertebra			Thickness at thirteenth vertebra		
	Lot 4	Lot 5	Lot 6	Lot 4	Lot 5	Lot 6	Lot 4	Lot 5	Lot 6
	Mm	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*
4	34	27	30	30	26	27	------	------	------
6	51	43	45	46	40	41	42	39	35

In the case of dairy animals on a low-phosphorus ration, it is impossible to keep the animal fat, for not only is the feed poorly utilized but it is impossible to induce the animal to eat sufficient amounts of the ration to store energy during the later stages of aphosphorosis. It was possible with the pigs in this study to take advantage of their increased thirst to force them to eat their feed in the form of a slop, and thus provide excess energy to be deposited in the form of fat, even though they were not utilizing their feed as fully as the normal pigs.

EFFECT ON THE GROWTH OF THE BODY AND THE DEVELOPMENT OF BONE AND MUSCLE

One of the more marked effects of the lack of phosphorus in the ration was a failure of the body to grow normally, and especially to develop bones and muscle to a normal extent.

It can be seen from the lower graphs in figure 1 that the hogs in experiment 1 receiving the low-phosphorus ration (lot 1), although

they received the same amount of feed and had the same initial weight, were about 17 percent lighter at the end of the experiment than those in lots 2 and 3. In experiment 2 there was a difference of about 25 percent in the weight of the pigs receiving a low-phosphorus ration (lot 4) and that of those on the higher phosphorus levels (lots 5 and 6).

Not only did the pigs on the low-phosphorus level weigh less (fig. 1, *C*, *D*), but their lengths (fig. 1, *A* and *B*, and fig. 2) and heights (figs. 1, *A*, *B*) were also less.

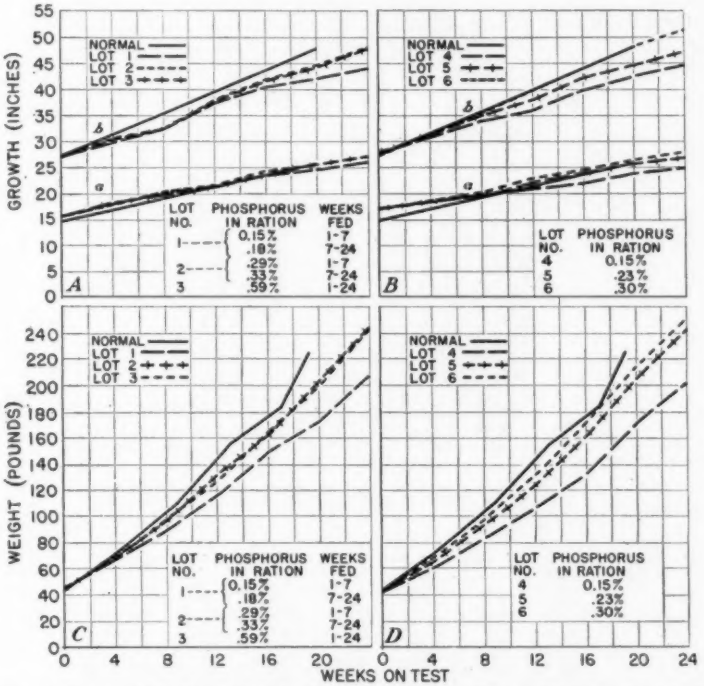

FIGURE 1.—Increases in the dimensions and weights of pigs on low-, medium-, and high-phosphorus intakes as compared with the dimensions and weights of normal pigs. Curves for normal animals were drawn from unpublished data supplied by the Division of Animal Husbandry of the University of Minnesota and represent the average of the records of 134 pigs of record-of-performance litters. The pigs in these litters were of all breeds and were self-fed on excellent rations. *A* and *B* show at *a*, height at shoulder, and at *b*, length from base of ear to root of tail; *C* and *D* show body weights. Lots 1 and 4 were on low-phosphorus, lots 2 and 5 on medium-phosphorus, and lots 3 and 6 on high-phosphorus rations, as explained in the text.

The lighter body weight was also correlated with poorly developed bone, as is shown in table 3. This table shows for pigs on the different levels of phosphorus, the variations in the bones, the specific gravity, breaking pressure, dimensions of the bone, and the ash content.

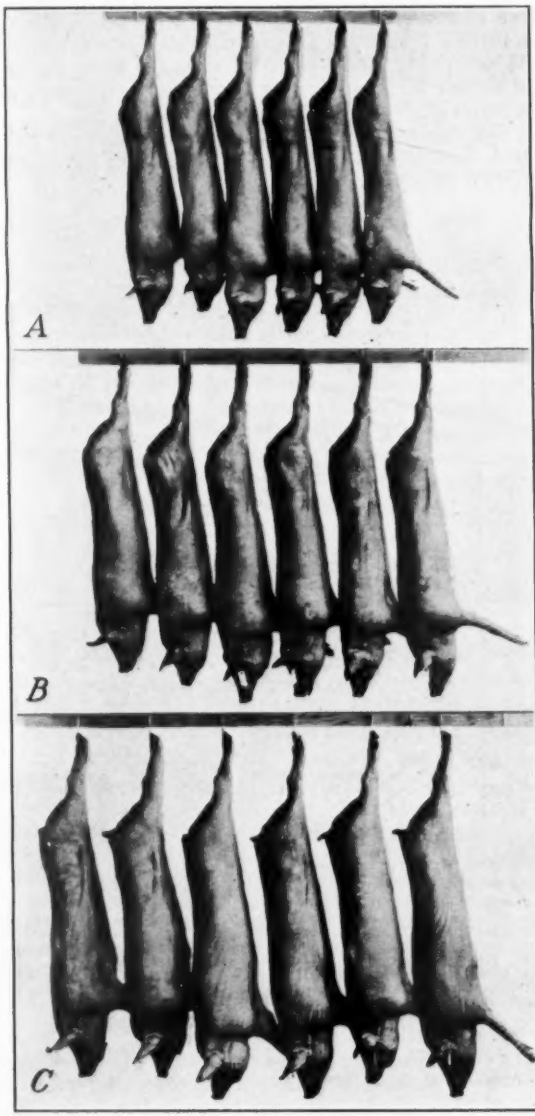

FIGURE 2.—Carcasses showing the development of the pigs in experiment 1: *A*, At the end of 8 weeks; *B*, at the end of 16 weeks; *C*, at the end of the experiment. Note the length and thickness of the carcasses. The two carcasses on the left in each group are from lot 1 (low-phosphorus); the next two carcasses in each group are from lot 2 (medium-phosphorus); and the two on the right in each group are from lot 3 (high-phosphorus).

TABLE 3.—*Average specific gravity, breaking strength, length, diameter, thickness of wall, and ash of the green leg and rib bones of two pigs*

TABLE 3.—*Average specific gravity, breaking strength, length, diameter, thickness of wall, and ash of the green leg and rib bones of two pigs in each lot, by 56-day periods in experiments 1 and 2*

EXPERIMENT 1

Period (28 days) no.	Bones	Specific gravity			Breaking pressure			Length of bones [1]			Diameter of bones [2]			Thickness of wall [3]			Ash [4]		
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
					Pounds	*Pounds*	*Pounds*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Percent*	*Percent*	*Percent*
2	Humerus	1.102	1.241	1.234	305	575	635	5.49	5.57	5.57	0.660	0.713	0.725	0.094	0.138	0.133	48.14	57.35	59.64
	Femur	1.098	1.233	1.223	190	595	590	6.31	6.52	6.46	.636	.647	.675	.103	.153	.169			
	Sixth rib	1.076	1.200	1.170	25	78	79	5.33	5.62	5.67							53.08	60.03	60.32
	Eleventh rib	1.091	1.226	1.155	16	64	65	5.18	5.47	5.58									
4	Humerus	1.124	1.221	1.237	420	745	910	6.57	6.78	6.67	.744	.825	.825	.120	.168	.184	52.72	59.63	61.08
	Femur	1.108	1.225	1.219	427	945	975	7.03	7.73	7.81	.745	.822	.797	.115	.191	.187			
	Sixth rib	1.042	1.159	1.193	61	157	157	6.67	6.97	7.16							52.29	58.80	60.97
	Eleventh rib	1.071	1.216	1.211	50	156	153	6.48	7.00	7.02									
6	Humerus	1.167	1.339	1.386	500	1,435	1,380	6.95	7.32	7.14	.852	.992	.877	.111	.206	.182	54.16	62.29	62.82
	Femur	1.167	1.338	1.345	585	1,490	1,455	7.72	8.41	8.23	.895	.937	.877	.086	.206	.179			
	Sixth rib	1.121	1.398	1.395	60	370	390	7.52	7.72	7.71							52.88	61.04	61.84
	Eleventh rib	1.193	1.483	1.455	55	345	345	5.53	7.73	7.62									

EXPERIMENT 2

Period (28) days) no.	Bones	Lot 4	Lot 5	Lot 6	Lot 4	Lot 5	Lot 6	Lot 4	Lot 5	Lot 6	Lot 4	Lot 5	Lot 6	Lot 4	Lot 5	Lot 6	Lot 4	Lot 5	Lot 6
2	Humerus	1.135	1.177	1.182	320	470	480	5.82	5.67	5.66	0.660	0.667	0.706	0.092	0.103	0.102	[5]	[5]	[5]
	Femur	1.122	1.164	1.171	330	540	550	6.51	6.46	6.36	.660	.663	.712	.087	.191	.135	[5]	[5]	[5]
	Sixth rib	1.079	1.116	1.166	29	75	78	6.24	5.96	6.11							[5]	[5]	[5]
	Eleventh rib	1.077	1.147	1.188	29	48	47	6.01	6.12	6.12							[5]	[5]	[5]
4	Humerus	1.173	1.205	1.184	440	755	897	6.40	6.65	6.90	.748	.836	.883	.107	.172	.165	[5]	[5]	[5]
	Femur	1.166	1.191	1.242	375	747	900	6.85	7.70	7.90	.732	.789	.797	.099	.152	.175	[5]	[5]	[5]
	Sixth rib	1.128	1.177	1.252	56	114	176	6.50	7.50	7.60							[5]	[5]	[5]
	Eleventh rib	1.196	1.204	1.318	46	86	154	6.50	7.30	7.45							[5]	[5]	[5]
6	Humerus	1.181	1.221	1.300	560	840	1,220	7.17	7.38	7.68	.824	.886	.932	.136	.176	.193	[5]	[5]	[5]
	Femur	1.171	1.221	1.294	590	1,030	1,310	7.72	8.46	8.80	.800	.892	.911	.130	.157	.180	[5]	[5]	[5]
	Sixth rib	1.079	1.177	1.284	65	160	295	7.17	8.08	8.48							[5]	[5]	[5]
	Eleventh rib	1.152	1.281	1.308	62	145	295	6.92	7.79	7.89							[5]	[5]	[5]

[1] Length over all of leg bones; ribs shortest distance from head to end of shaft.
[2] Average of smallest and greatest diameter at breaking point.
[3] Average of smallest and greatest thickness of wall at breaking point.
[4] Average of the 2 leg or 2 rib bones.
[5] Determinations lost in the burning of the chemistry building.

The ribs and vertebrae of the pigs fed low levels of phosphorus showed poor calcification similar to that usually observed in animals suffering from low-phosphorus rickets. The poor development of bone is also indicated by the fact that as early as 7 weeks after the beginning of the experiments the legs of the low-phosphorus pigs were somewhat deformed. This condition became more marked as the experiments progressed, so that by the end of the experiments the pigs had difficulty in moving about in their pen.

It is rather to be expected that the growth of bone, which contains a high percentage of calcium and phosphorus, would be materially influenced if the phosphorus were not provided in the feed. It is not generally thought, however, that this mineral plays such an important part in the development of the muscle, because the percentage of ash in muscle is comparatively low. When it is considered that phosphoric acid is an integral part of the nuclei of the cells, which have much to do with the cell development, and that the phospholipins play an essential part in the function of the muscle fiber, it does not seem impossible that a deficiency in phosphorus would be reflected in decreased muscle development.

EFFECT ON THE INORGANIC PHOSPHORUS OF THE BLOOD

The results of the effect of low-phosphorus rations on the inorganic phosphorus of the blood (table 4) are in accord with the results reported by other investigators for swine.

TABLE 4.—*Average calcium and inorganic-phosphorus content of the blood of pigs by 28-day periods in experiments 1 and 2*

EXPERIMENT 1

Period no.	Pigs	Calcium (per 100 cc of serum)			Inorganic phosphorus (per 100 cc of whole blood)		
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
	Number	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Initial[1]	6	11.9	11.9	11.7	4.3	5.5	6.6
1	6	12.8	12.3	11.1	3.9	4.9	6.1
2	6	13.3	12.4	11.8	3.4	4.8	5.4
3	4	13.7	12.9	12.3	3.7	4.8	5.9
4	4	14.9	12.8	12.1	2.9	4.8	6.1
5	2	13.3	12.1	12.4	3.2	5.1	6.0
6	2	12.9	12.1	11.6	3.3	5.8	6.8

EXPERIMENT 2

Period no.	Pigs	Lot 4	Lot 5	Lot 6	Lot 4	Lot 5	Lot 6
	Number	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Initial	6	9.7	10.0	10.0	5.5	5.7	5.7
1	6	12.4	11.2	11.2	4.1	5.0	5.7
2	6	12.6	12.0	12.1	3.4	4.7	5.5
3	4	11.0	10.6	10.3	2.9	4.7	5.5
4	4	11.9	12.1	11.0	2.7	4.8	5.3
5	2	11.5	12.0	11.1	3.5	5.1	5.5
6	2	13.2	13.7	11.5	2.6	5.4	6.2

[1] The blood samples for this analysis were taken about 2 hours after the second feed of the experimental ration had been given the pigs.

An inspection of these data clearly shows that the pigs in lots 1 and 4 quickly developed a state of phosphorus deficiency, which became more marked as the experiment progressed. Lots 1 and 4 had the least amount of inorganic phosphorus in the blood. Lots 3 and 6 had the highest percentage, while the amount in lots 2 and 5 were between that of the other two lots. Since each lot received a different level of phosphorus in its ration and since each lot had a distinctly separate level of inorganic phosphorus in the blood, it is clearly evident that the amount of phosphorus in the feed readily reflects itself in the blood.

This fact is shown further by the initial analysis of the blood of the pigs in experiment 1 (table 4). Through an oversight at the time of starting the experiment, blood was not drawn for analysis until after the second feed of the experimental ration had been consumed. In this short time the amount of phosphorus in the two feeds affected the inorganic phosphorus of the blood of the pigs in the three lots, so that three distinct levels resulted, corresponding to three definite levels in the rations.

EFFECT ON THE CONSUMPTION OF WATER AND THE EXCRETION OF URINE

The literature, so far as the authors have observed, does not mention an increased thirst as one of the symptoms of aphosphorosis. However, the data for a digestion trial with dairy cows reported by Riddell, Hughes, and Fitch (*8*), in which the consumption of water was accurately measured, showed that the average amount of water consumed by the two low-phosphorus cows was about 4 percent more than consumed by the cows receiving the same ration supplemented with phosphorus. The low-phosphorus cows also excreted 27.2 percent more urine. However, no attention was called to these variations in the discussion of this digestion trial.

In experiment 1 it was noticed as early as the second week that the pigs of lot 1 were consuming considerably more water than the pigs in the other lots receiving a higher level of phosphorus. Accordingly, a check of the consumption of water of the pigs was made for 2 days of each month. The results are shown in table 5.

TABLE 5.—*Water consumed daily by and weight of kidneys of pigs in experiment*[1]

Period (28 days) no.	Pigs	Water consumed daily [1]			Weight of kidneys [2]		
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
	Number	*Kilograms*	*Kilograms*	*Kilograms*	*Grams*	*Grams*	*Grams*
1	6	4.77	3.68	3.31			
2	6	9.09	7.13	5.59	220	177	185
3	4	8.95	8.22	6.63			
4	4	11.18	10.13	8.27	302	264	230
5	2	(3)	12.31	11.45			
6	2	(3)	15.86	14.13	395	280	275

[1] Average per pig calculated from the water consumption for 2 days of each month.
[2] Average for 2 pigs in each lot.
[3] No data were secured for lot 1 for periods 5 and 6.

While it was apparent that more urine was being excreted, no measure was made of it in this experiment.

Because the pigs exhibited excessive thirst and urination, special attention was given to the weight and appearance of the kidneys at the time of slaughter. Table 5 shows that the kidneys of the pigs on the low-phosphorus ration were larger than those from the pigs receiving the higher levels of phosphorus. It is also apparent that the differences in the weights of the kidneys became more marked as the experiment progressed.

During the second experiment it was possible, by placing the pigs in a metabolism crate, to measure not only the water consumption but the excretion of urine for a 3-day period.

The water consumption was 11,514, 6,190, and 5,903 g, and the urinary excretion was 8,541, 2,300, and 2,355 g for lots 4, 5, and 6, respectively. The average weights of the kidneys for lot 4 was 411 g, for lot 5, 366 g, and for lot 6, 335 g.

Thus the increased thirst and the increased urinary excretion of the pigs on the low-phosphorus level were evident again. The kidneys also were larger in the low-phosphorus-fed pigs at the close of this experiment.

Histological examination of the enlarged kidneys from the lots of pigs maintained on the low level of phosphorus disclosed that they were distinctly enlarged and light in color, presenting the appearance of a "large white kidney." Microscopically this organ showed evidence of a chronic diffuse nephritis of the parenchymatous type, and presented widened glomerular spaces around the glomerular tufts and also widely distended uriniferous tubules with flattened epithelial cells. A granular debris was present in some of the tubules.

It is significant that the impaired kidney corresponded with the excessive urination and accompanying thirst observed in these experiments.

SUMMARY

The results are reported of an investigation of the effects of the feeding of different levels of phosphorus in the rations of 36 young pigs.

Two experiments, each involving three lots of six pigs each, were carried on for 24 weeks.

Data are presented to show the effect of deficient amounts of phosphorus in the ration on the growth and development of pigs.

The results obtained indicate that the abnormalities resulting from feeding low-phosphorus rations were: (1) A loss of appetite, (2) a poor utilization of feed and storage of energy, (3) a failure to make normal growth and to develop bone and muscle normally, (4) a lowering of inorganic phosphorus in the blood, and (5) a marked increase in thirst and a corresponding excretion of urine.

LITERATURE CITED

(1) Bethke, R. M., Edgington, B. H., and Kick, C. H.
1933. effect of the calcium-phosphorus relationship of the ration on growth and bone formation in the pig. Jour. Agr. Research 47: 331–338, illus.

(2) Clark, E. P., and Collip, J. B.
1925. a study of the tisdall method for the determination of blood serum calcium with a suggested modification. Jour. Biol. Chem. 63: 461–464.

(3) Dunlop, G.
1935. the calcium, phosphorus, and vitamin d requirements of swine. Jour. Agr. Sci. [England] 25: [22]–49, illus.

(4) Eckles, C. H., Becker, R. B., and Palmer, L. S.
1926. a mineral deficiency in the rations of cattle. Minn. Agr. Expt. Sta. Bul. 229, 49 pp., illus.

(5) ——— Gullickson, T. W., and Palmer, L. S.
1932. phosphorus deficiency in the rations of cattle. Minn. Agr. Expt. Sta. Tech. Bull. 91, 118 pp., illus.

(6) Hankins, O. G., and Ellis, N. R.
1934. physical characteristics of hog carcasses as measures of fatness. Jour. Agr. Research 48: 257–264, illus.

(7) Malan, A. I., Green, H. H., and du Toit, P. J.
1928. studies in mineral metabolism. v. composition of bovine blood on phosphorus deficient pastures. Jour. Agr. Sci. [England] 18: [376]–383.

(8) Riddell, W. H., Hughes, J. S., and Fitch, J. B.
1934. the relation of phosphorus deficiency to the utilization of feed in dairy cattle. Kans. Agr. Expt. Sta. Tech. Bull. 36, 54 pp., illus.

(9) Theiler, A.
1920. the cause and prevention of lamziekte. Union So. Africa Dept. Agr. Jour. 1: 221–244.

(10) ——— and Green, H. H.
1932. aphosphorosis in ruminants. Nutrition Abs. and Rev. 1: 359–385.

(11) Youngburg, G. E., and Youngburg, M. V.
1930. phosphorus metabolism. i. a system of blood phosphorus analysis. Jour. Lab. and Clin. Med. 16: 158–166.

U. S. GOVERNMENT PRINTING OFFICE : 1936

XENOPHOBIA